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PRESIDENTIAL ADDRESS

FIGHT THE WHEAT RUST*

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The importance of wheat in world economy can not be over emphasized. In total production it is second to none taking world as a whole. In India wheat crop occupied an area of 30.966 million acres in 1958-59 yielding 9.69 million tons of grain. This works out to an average production of about 9 mds. per acre, which is very low as compared to other wheat producing countries. Where as this low yield has been variously ascribed to poor land, paucity of artificial fertilizers, poor irrigation facilities, unimproved seed, primitive agricultural practices, etc., quite a substantial harvest is lost to the diseases which afflict this crop year after year. In this country, as elsewhere, rusts occupy an important place amongst these diseases and cause considerable loss to the nation every year. Although accurate figures for the food grain lost annually to rusts have not been systematically determined on a country-wide basis by well-planned crop cutting experiments, small scale laboratory trials have shown that in Agra local wheat which is an un-improved "*Desi*" wheat there is a loss of 14.2% and 19.6% in grain weight with 10–25% and 40–50% rust infection, respectively. Corresponding figures for N.P. 4 were 7.9% and 13.1%. Since most of our land is still under unimproved local wheats, it would be no exaggeration to put the normal annual loss to 10%. Calculating on that basis nearly 1 million tons were lost in 1958–59 on account of depradations of rusts, which works out to a loss of Rs. 392 million at the rate of Rs. 14/- per maund, the Government controlled rate. Although these figures for annual losses have been mentioned by different workers on rusts several times before also, no apology seems to be necessary for repeating the fact again and again considering the national importance and the urgency of the problem. When it is borne in mind that during epidemic years, as happened only 3 years ago in Bihar, only ½ md. per acre was obtained, and that ½ md. too was no good even as cattle feed, the figures of losses calculated above would have to be multiplied several folds, the absolute urgency with which steps should be taken to combat the menace of rusts, with all the weapons at our command, could only be neglected at our perpetual peril.

All three rusts of wheat viz. Black, Brown and Yellow, caused by *Puccinia graminis tritici* (Pers.) Erikss. and Henn., *P. triticina* Pers. and *P. glumarum* (Schm.) Erikss. and Henn. occur in different parts of the country. In this respect, we are much worse off than most of the other countries where only one or the other rust is of primary importance. Over the greater part of the country, wheat is a winter crop, sown in October-

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November and harvested in March-April. During the off-season, there is no wheat or any other congenial host in the plains and the summer is so hot as to make it impossible for the rust to survive. Observations extending over a period of nearly 35 years have conclusively proved the over-summering of rusts on self-sown plants, tillers etc. in general at suitable altitudes in the hills above 3,000 ft. a.s.l. and on summer crop in restricted areas like Nilgiri and Palni hills. Although several wild grasses have been shown to be susceptible to one or the other of these rusts under artificial infection, as yet there is no evidence to prove that they function as natural agents in their perpetuation from one crop to the next. In the case of alternate hosts too, there is no evidence to show that they are functional. Since 1930, sixteen physiologic races and biotypes of black rust, 12 of brown rust and 10 of yellow rust have been identified on standard and supplementary differential hosts. These races have not shown any mutation in pathogenicity even when most of them have been more than 25 years in greenhouse cultures. From the data available it is obvious that the race flora has shown considerable variation and prevalence of some of the races has undergone wide fluctuation. Race 15 of black rust which was the most common race before 1938 has almost disappeared, whereas race 21 which, after being identified from one place in 1933-34 was not met with at all till 1942, subsequently became the most prevalent race. In recent years several new races have been identified. Such examples of shift or changes in racial population are not rare and have been reported from several other countries although the precise reasons to account for them are not clear. They could be due to changes in wheat varieties under cultivation or a combined effect of a large number of ecological factors or both. Work on the factors responsible for changes in race flora is in progress at the Indian Agricultural Research Institute and there is evidence to show that "race-variety relationship" governs the survival of races in mixtures which may also be affected by certain environmental conditions e.g. light, temperature and humidity. Besides such shifts in race populations, the appearance of new races is a factor of great significance in breeding for resistance. New races are known to arise through hybridization, mutation and introduction from neighbouring countries. To these may now be added the recent discovery of heterocaryosis by Nelson *et al* and of somatic hybridization by Watson, who obtained new races by mixing uredospores of known races. Watson felt that new races were coming up in Australia by some such process in the absence of the alternate host. Subsequent to my observation of infected barberries in Tasmania for the first time in 1956, Watson has confirmed that some of these infections are connected with wheat black rust and may atleast occasionally account for the introduction of new races into the main land.

Which of the above-mentioned factors is operative in producing new races that have been picked up by us in recent years is not clearly understood. So far there is no evidence of hybridization or mutation. Information from neighbouring countries like Afghanistan and Iran is meagre, so that nothing could be said with certainty whether new races are being introduced from those areas. Further work will alone confirm whether heterocaryosis or somatic hybridization are active under natural conditions. If they were, a very large number of new races should have come into being even in those tracts where the alternate hosts do not exist.

Another factor which might account for the identification of several new races in recent years is the intensification of and increase in the number of samples for this study. Even now, not more than 500 collections of all the three rusts taken together can be analysed with the available facilities of man power and greenhouse accommodation. This number of collections is highly inadequate for a crop occupying nearly 31 million acres, and chances of missing the existing races are very great. In other words, what we call new races may be only new finds.

From what has been said above it would be clear that work should be intensified on survey in the hills to discover pockets of barberry infection, if any. Although barberries do not play any part in the annual recurrence of the rust in India, nevertheless, they may be instrumental in creating new races by hybridization. In that case, it would become necessary to eradicate them from such pockets of infection. This survey should also include search for wild grasses for their infection with wheat rusts. Additional facilities should also be provided to study a much larger number of rust collections for the identification of physiologic races and biotypes, so that the plant breeder can proceed with his work of evolving resistant varieties with greater confidence. Even if one collection of black rust is studied for every 10 thousand acres, the total number for black rust alone would come to more than 3 thousand. To this should be added an equal number for the other rusts.

Methods of Control: With the present knowledge of the annual recurrence of rusts in India where perpetuation takes place through the agency of uredospores in the hills, Mehta had suggested that rust epidemics could be controlled to a considerable extent by reducing the initial inoculum at the source. To achieve that he emphasized the importance and urgency of breeding rust resistant varieties and gave priority to the hills which act as foci of infection. Till such time as suitable resistant varieties are available and hilly areas saturated with them, he advocated the clean-up of volunteer wheat and barley plants and suspension of summer crop in Nilgiri and Palni hills, as also the replacement of wheat and barley cultivation by that of oats in the hills.

As pointed out by Chester "Adjustment of the time of sowing, fertilization, destruction of volunteer cereal plants, application of fungicides—these are but adjuncts to the fundamental solution of the cereal rust problem : the breeding of cereal varieties that are resistant to rust, — at once the most certain and effective, and the most economical means of checking the ravages of the cereal rusts."

In India systematic work on the breeding of rust resistant wheats was started by Pal in 1935 in collaboration with Mehta and several rust tolerant varieties have by now been released for general cultivation. These varieties have done very well in different States and given much higher yield than the local varieties even under epidemic conditions. That different varieties do not suffer in their yield to the same extent with equal amount of rust infection has already been indicated. In controlled experiments Gokhale and Patel showed that the loss in grain weight did not

exceed 30 per cent in N.P. 710 and N.P. 715, and 50 per cent in N.P. 52, whereas it was almost 100 per cent in Motia and A. 013. In 1956-57 Bihar wheat rust epidemic the entire crop was destroyed by the fungus and only ½ md. per acre yield was obtained from local varieties. Under identical conditions those cultivators who had grown N.P. 797, N.P. 798 and N.P. 799 reported as much as 12 mds. yield per acre. These varieties had thus demonstrated conclusively their superiority even under epidemic conditions.

It may, however, be pointed out that these N.P. wheat varieties are capable of giving even better performance, since in normal years of light rust infection their average yield in Bihar is as high as 15–18 mds. per acre. This means that the yield of even the improved varieties might be knocked off by 20–25 per cent under epidemic conditions. My plea is that something should be done to save this loss of precious wheat. The resistant varieties should be protected from the ravages of rust spores by reducing the inoculum at the foci of infection and at the infection court so that they can come up to their best. In recent years interest has developed in evolving suitable fungicides and considerable amount of information is available in literature on this subject.

The control of rusts by chemicals is not a new idea and dates back to atleast 1900. Since then the efficacy of sulphur was proved in the field in Canada and it was recommended that 3 to 5 applications at the rate of 30 lbs. per acre per application would control stem rust and increase the yields from 25 to 100 per cent depending on the severity of rust. The cost involved was, however, prohibitive and was not commensurate with the gain. Also, enormous quantities of sulphur were required. The use of sulphur in the control of rusts on a wide scale, therefore, has never been recommended.

Realisation that rust resistant varieties may not continue to be resistant permanently on account of shifts in physiologic races has revived interest in chemical control. Search for chemicals that would be effective in small quantities has lead to the formulation of such fungicides as Dithane, Zineb and Actidione. Extensive tests in different countries indicate that rust can now be controlled economically with the help of these compounds provided that weather conditions do not become very adverse during these operations. Dithane has been used successfully and economically in the control of rusts in Japan. Four to five applications of Nabam and Zinc sulphate gave effective control of wheat rusts at Winipeg and the cost of the chemicals was about ⅓ that of sulphur. At Wyoming Agricultural Experiment Station, 2 applications of Actidione reduced infection from 25 to 5 per cent and increased the yield 1½ times of the untreated. Actidione is a promising antibiotic in the control of rusts as a systemic fungicide. Greenhouse experiments at the University Farm, St. Paul, Minn. demonstrated that certain sulpha compounds including sodium salts of sulphadiazine, sulphapyrazine, sulphapyridine and gantrisin are effective against black rust even as post-infection sprays. Certain naphthoquinones and phenols exert a fungicidal action at low concentrations on cereal stem rust as reported from Wisconsin Agricultural Experimental

Station. At Nebraska Agricultural Experiment Station calcium sulphamate gave excellent results in the field but induced deleterious changes in the harvested seed which impaired its baking properties. Actidione and calcium sulphanilate were effective against the rusts and exerted no adverse effect on the quality of the flour. Calcium sulphamate controls rust by inactivating the fungus within the host tissue. Recently, Parzate liquid with Zinc sulphate has been found to be effective in reducing rust infection appreciably under artificial epiphytotics at Indian Agricultural Research Institute It is not my intention to give an exhaustive review of the subject and these are only some of the results obtained in different countries to show the economic possibility of controlling rusts with the help of fungicides.

Side by side with the breeding of resistant varieties, there is need for extensive basic research on chemical methods of rust control, which is independent of the physiologic races present in epiphytotic years. The two i.e. breeding for resistance and chemical protection must make parallel contribution in order to get the best out of improved varieties. It is much more economical and effective to use the fungicide on resistant varieties than on local varieties. The former can be protected with fewer applications and consequently the economics of the treatment would be favourable. In a way, this country is more favourably placed with respect of wheat rust control since the foci of infection are circumscribed and restricted to wheat areas in the hills, which form less than 5 per cent of the total acerage under this crop in the country as a whole. Supplementing the introduction of resistant varieties with their chemical protection in the hills alone would go a long way in preventing serious rust epidemics both in the hills as well as the plains, although operational difficulties presented by the terrain can not be overlooked. With greater emphasis on Community Development Projects and Cooperative Farming, wide scale spraying or dusting of fungicides with the help of power machines will become a practical and economic possibility. For an effective fungicidal control programme, an efficient disease-forecasting service is essential, so that preventive measures are taken in good time and most economically. Gentlemen, we must fight this 'shifty enemy' on all fronts and with every weapon. Then and then alone there is a reasonable prospect of victory. In victory over rusts most of our battle over the food front would have been won.

STUDIES IN USTILAGINALES
3. MORPHOLOGY, CYTOLOGY AND SPORE GERMINATION OF ENTYLOMA DAHLIAE ON DAHLIA VARIABILIS DESF.

N. C. Joshi

(Accepted for publication December 15, 1959)

The paper summarises the results of study by the author on the morphology, cytology and spore germination of *Entyloma dahliae* which causes an epiphytotic on *Dahlia variabilis* in Almora and Mussoorie hills, of U. P.

Material and Methods. *Entyloma dahliae* was collected from Almora and Mussoorie on *Dahlia variabilis* Some affected leaves were first dried and then kept in the cellophane bags for the spore germination studies. Some freshly infested material was also fixed in F.A.A., Flemming's solution and 70% alcohol at various stages for studying the nuclear condition and the mode of spore development inside the host. Microtome sections of 6-12 μ thickness were cut and stained with Flemming's triple stain and iron alum haemotoxylin. The latter gave the best results so far the nuclear details were concerned. (For spore germination studies salts, sugars and other nutrients were obtained in a pure state. The pH of the solution was determined by Beckman's pH meter).

The disease is commonly called leaf spot and only a preliminary account of this disease has been given by Pethybridge (1928) and Sydow *et al* (1912). The symptoms are mainly found on the leaf. In earlier stages small patches of yellow green colour were observed on the leaf. In majority of the cases these patches were circular while in some they were angular in shape (Fig.1). As the time advanced these spots turned brown and ultimately grey in colour. Sometimes they developed necrotic spots and produced many lesions and perforations; in several cases one or two spots coalesced and produced larger spots and ultimately the whole leaf gets shrivelled and destroyed.

Histopathology. The infested tissue of the leaf does not show clear differentiation into the palisade and spongy parenchyma (Fig. 2). In majority of the cases the palisade cells lose their identity. The contour of the nucleus becomes irregular. The chloroplast decomposes, dimnishes in size until finally disappears. The vascular tissue is not affected by the parasite.

Spore Germination. A preliminary account of the spore germination of *Entyloma dahliae* is given by Pape (1926), Pethybridge (1928) and Green (1932). The writer studied the spore germination in detail which is given below:

Fig. 1. Showing the infected leaves of *Dahlia variabilis* by *Entyloma dahliae*.

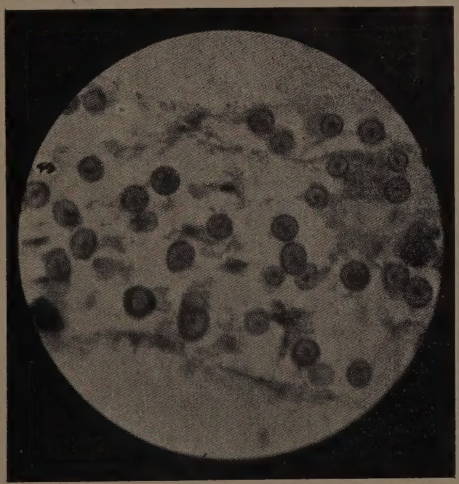

Fig. 2. Photomicrograph of the transverse section of the leaf of *Dahlia variabilis* infected by *Entyloma dahliae* showing the morbid condition of the cells. The chlamydospores lying in the intercellular spaces. x 500 times.

The spore germinated readily in water and other nutrient solutions. At the time of germination the exosporium ruptures at one point, the endosporium protrudes out and gives rise to the promycelium. The latter varies from 60—80 μ in length and 5-8μ in breadth. At the tip of the promycelium protuberances appear which are very conspicuous in the later stage and are called sporidia. The sporidia produced at the tip of the promycelium are acicular and measure 40-60 μ in length and 3-5 μ in breadth. Soon after two sporidia fuse resulting into the formation of a curved cunjugation tube through which the nucleus of one sporidium travels into the other (Fig. 3). Sometimes the sporidia do not fuse and uninucleate secondary sporidia are formed (Fig. 4). The dicaryotic stage is obtained by the union of two sporidia.

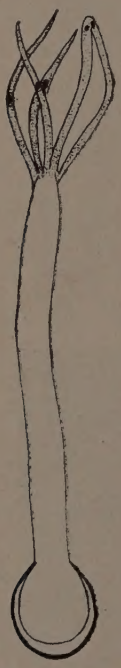

Fig. 3. Formation of the conjugation tube by the fusion of two sporidia. x 1500 times.

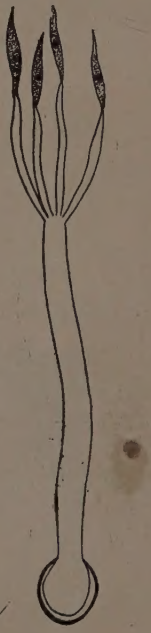

Fig. 4. Formation of the secondary sporidia at the tip of the primary ones. x 1500 times.

Effect of Salts on Spore Germination. The spores were germinated at room temperature (i.e 22-23 °C.) in 0.1 to 1 per cent solutions of sodium chloride, sodium carbonate, ammonium sulphate, potassium chloride, potassium sulphate, magnesium sulphate, potassium nitrate and potassium phosphate. The salt solutions did not exert a favourable influence on spore germination and percentage of the germination was not more than 18. It was noted that sodium carbonate and sodium chloride were

toxic and there was no germination even at 0.1 per cent solution of these salts. Ammonium sulphate and potassium chloride were toxic but not as much as sodium chloride and sodium carbonate. In potassium sulphate, magnesium sulphate, potassium nitrate and potassium phosphate germination was fairly low (i.e. not above 12 per ent). In potassium nitrate the germination was 18 per cent.

Effect of sugars on spore germination. The spores were germinated in 5 per cent solution of dextrose, levulose, maltose, lactose, sucrose, galactose and malt extract. Malt extract was best and the percentage of germination was 45.

Effect of nutrients other than sugars on spore germination. The spores were germinated at room temperature (i. e. 22-23 C.) in the nutrients other than sugars i.e. Dahlia leaf extract, soil extract, dung extract, yeast extract, distilled water and beef extract. It appeared that the germination percentage was more in nutrient solutions than in distilled water. Dung extract gave the best results (i. e. 42 per cent).

Effect of hydrogen on concentration. Further observations were made on the effect of pH when the spores were germinated in 5 per cent malt extract solution at room temperature (i.e. 22-23°C.) having the pH range of 1.5 to 10.5 which was determined by Beckman's pH meter. It was noted that the spores were capable of germination in a wide range of pH, i. e. 2.5 to 10. The optimum germination being at pH 5.5 while there was no germination below 1.5 and above 10.5

Effect of temperature on spore germination. The spores were germinated in 5 per cent glucose solution at temperatures ranging from 5 to 40°C. The optimum temperature for spore germination is about 25°C, below 5°C. and above 40°C. there was no germination.

Discussion. The chlamydospores of *Entyloma dahliae* which contain a diploid nucleus like other smuts, vary in size. The diameter of spores was 11-17 μ according to Green (1932); 12.5-16 μ according to Flach (1927) and 10-16 μ according to Pape (1926) but the author who collected this smut from Almora and Mussoorie noted that they measured 11-15.2 μ in diameter. The spores of this smut germinated readily in water and other nutrient solutions unlike the spores of *Entyloma microsporum* (Das 1947) and *Tilletia holci* (Das 1948). At the time of spore germination the division of the diploid nucleus takes place in the promycelium. In this respect it resembles very much with *Tilletia tritici* (Holton & Heald 1941). The growth of the promycelium varies. Soon after the promycelium bears a cluster of 4-6 sporidia at its tip. The fully developed sporidium measures 40-60 μ by 3-5 μ in dimensions. The measurements made by Pape (1926) and Green (1932) were 45-75 μ by 2 μ and 60 by 1 μ respectively. The origin of dicaryotic stage in this fungus is achieved by the union of two sporidia as in *Tilletia tritici* (Dastur 1921).

SUMMARY

Entyloma dahliae causes greyish brown spots on the leaves of *Dahliae variabilis* and causes an epiphytotic in the hills (i. e. Almora and Mussoorie) in Uttar Pradesh.

The fresh chlamydospores germinated within 24 hours in water and other nutrient solutions. On germination a long aseptate promycelium with 4-6 sporidia at its tip is given out. The sporidia measure 40-60 μ by 3-5 μ. At the time of germination of the spores the diploid nucleus migrates into the promycelium and there the division takes place, the conjugation tubes are developed between two sporidia. The dicaryotic stage is obtained by the union of two sporidia.

ACKNOWLEDGMENT. I am grateful to Dr. M. J. Thirumalachar for suggesting the problem. I am also thankful to Dr. G. W. Fischer, Professor of Plant Pathology, State College, Washington and Dr. D. B. O. Savile of Canada for sending the reprints of their papers for this work. My thanks are also due to Principal Bhim Sen and Prof. Tiagi for the facilities.

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FACTORS AFFECTING THE GROWTH OF COLLETOTRICHUM CAPSICI

A. P. Misra and M. Mahmood

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The 'Die-back' of chillies caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is probably the most serious disease of chillies in Bihar, and also occurs in Madras, Assam and other States. It was first reported by Sydow from Madras Presidency in 1913. Dastur (1921) and Chowdhury (1957) later reported it to be serious in Bihar and Assam respectively. Studies on the morphology and physiology of the fungus were carried out by Dastur (1921), Chowdhury (1957) and Thind and Randhawa (1957). In the present studies the effects of several factors on spore germination, growth and sporulation of the fungus were investigated.

Material and Methods. The fungus was isolated from diseased chilli plants and brought into pure culture by dilution method and by the transfer of a single germinating spore. Spore germination studies were made in hanging drops, using Van Tiegham cells, and the effects of different temperatures, light intensities, pH, atmospheric humidity and different media were investigated, using conidia from seven day old cultures.

For cultural studies the fungus was grown in various synthetic and non-synthetic media and incubated at 30 °C. The radial growth of the colony and the weights of mycelial mats were recorded at intervals of 24 hours and 21 days respectively.

Experimental Results

Spore Germination Studies. The conidia germinated readily in tap water and host extract within 4 hours, at 30 °C. Germination was maximum at this temperature, and decreased at lower and higher temperatures; at 7.2 °C. no germination was observed. Light had no effect on germination. Of several media tried, the germination was maximum (93.3 per cent) in 1 per cent sucrose solution, although the percentages were quite high in almost all other media, viz., water, leaf decoction, leaf extract, 0.5 per cent agar solution and 0.5 per cent agar plus 1 per cent sucrose solution. Conidia germinated in 100 percent atmospheric humidity in water, below this humidity no germination was observed.

To study the effect of different hydrogen ion concentrations on spore germination, conidial suspensions were made in Czapek's solution containing 1 per cent sucrose and adjusted to different levels of pH and incubated at 30 °C. for 24 hours. Maximum germination of conidia took place in neutral solution. Above and below neutral levels the percentages of spore germination decreased and was nil at pH 9.7.

The growth rates of germ tubes were also studied on different media at different temperatures. The growth of germ tubes was maximum in leaf extract and this was followed in order by leaf decoction, 1 per cent sucrose solution and water. The growth of germ tubes was faster at 30°C. than at 20°C.

GROWTH AND SPORULATION OF *C. capsici* ON DIFFERENT SOLID MEDIA. Seven different synthetic and non-synthetic media were tried. Observations were made on mycelial growth, sporulation and size of conidia and setae, obtained from two week old cultures. Data are presented in Table 1.

TABLE 1. Effect of different media on growth and sporulation of *C. capsici*.

Media	Average radial growth in mms. after 6 days	Sporulation	Average length of conidia in μ	Setae in μ
Richard's agar	86.6	Nil	Conidia and setae not formed	
Czapek's agar	79.3	Abundant	22.54	155.21
Brown's agar	69.3	Nil	—	—
Potato dextrose agar	70.0	Abundant	25.34	251.26
Oat meal agar	68.3	Moderate	22.46	178.71
Corn meal agar	62.6	Moderate	24.0	193.94
Host extract agar	58.0	Abundant	23.16	131.51

It will be observed from the data presented above that amongst the synthetic media the mycelial growth was most profuse in Richard's medium, whereas, sporulation was most abundant in Czapek's medium. Amongst non-synthetic media, potato dextrose agar was best, both for mycelial growth and sporulation. The size of conidia and setae was also maximum on this medium.

The cultural characters in case of potato dextrose and corn meal agar media were similar to those described by Dastur (1921) and Ling and Lin (1944). In other media the main points of difference were as follows:

In Richard's medium the growth was both submerged and aerial in early stages, but later on it became completely aerial, profuse and compact. No acervuli were formed. In Czapek's agar medium also the growth was compact, aerial and fairly profuse, but in contrast to the Richard's medium abundant acervuli were formed, which were scattered all over the medium from the periphery to the centre. In Brown's agar medium, the mycelium at first aerial and loose, was submerged in later stages and covered the surface of the medium like crepe paper and no acervuli were formed. In Oat meal agar medium, the mycelial growth was aerial, cottony and loose,

and the acervuli were scattered. In contrast to the growth pattern in potato dextrose agar, no zonation was produced. In Host extract agar medium. the mycelial growth was less in comparison and the acervuli were arranged in rings, the colony later on submerged.

GROWTH AND SPORULATION IN DIFFERENT LIQUID MEDIA. The fungus was also cultivated on different liquid media. Observations on dry weight of the mycelium and sporulation after 15 days were as follows:

Media	Average mycelial weights in mgms.	Sporulation
Richrd's medium	402.2	Nil
Czapek's medium	233.2	Abundant
Brown's medium	35.7	Nil

The main cultural characters were as follows:

In Richard's medium the mycelial growth was thick, compact and cream coloured and the medium colour turned yellowish, no acervuli were formed. In Czapek's medium the mycelial growth was less thick, but pinkish or brownish spore masses, as well as acervuli were abundant. In this case also the medium colour turned yellowish. In Brown's medium the growth was poor and without acervulation and the medium remained colourless.

The importance of the different constituents of Czapek's medium, which was taken as the standard medium was also investigated. The omission of either Sucrose, Potassium dihydrogen phosphate, Magnesium sulphate or Sodium nitrate markedly retarded mycelial growth. The omission of these salts and Ferrous sulphate also affected sporulation, which was nil in the absence of Surcose, Potassium dihydrogen phosphate, magnesium sulphate and Ferrous sulphate and developed in traces only in the absence of Sodium nitrate. Data are presented in Table 2.

TABLE. 2. Growth and sporulation of *C. capsici* in the absence of either of the constituents of Czapek's medium.

Constituents lacking	Average dry weight in mgs.	Sporulation	Percentage of increase or decrease in dry weight
Magnesium sulphate	110.3	Nil	–52.1
Potassium dihydrogen phosphate	74.0	Nil	–67.8
Potassium chloride	264.6	Moderate	+14.8
Ferrous sulphate	246.3	Nil	+6.9
Sodium nitrate	117.0	Traces	–49.1
Sucrose	35.0	Nil	–89.1
Control (Complete Czapek's medium)	230.3	Abundant	

Effect of temperature on mycelial growth and sporulation of the fungus. The effect of temperature on the rate of growth of *C. capsici* was also studied. Petri dishes containing equal volumes of potato dextrose agar were inoculated and incubated at 7.2, 15, 20, 25, 30, 32.5 and 35.5°C. Observations were made at intervals of 24 hours. The minimum temperature for the growth of the fungus lay between 7.2°C. and 15°C. and the optimum was 30°C. No growth occured at 7.2°C., and only slight growth occurred at 35.5°C.

Effect of light on growth and sporulation. To study the effect of light, one set of inoculated plates of potato dextrose agar was placed in open in the laboratory and the other set was placed under bell jar covered by black paper. A third set was exposed to continuous light of a 100 watt electric bulb in a dark room, alongside of which another set of plates was placed in complete darkness under bell jar covered with black paper. It was observed that alternate light and darkness were better than continuous darkness or light, both for growth and sporulation. The average growth of the colony and sporulation on the sixth day were as follows: Treatment differences were highly significant.

	Average growth in mms.	Sporulation
Continuous light	48.6	Sparse
Continuous dark (but with a 100 watt bulb in the chamber outside)	60.6	Sparse
Alternate light and darkness	72.0	Abundant
Continuous darkness	63.6	Sparse

Effect of Relative humidity on growth and sporulation. The fungus was grown on potato dextrose agar medium and exposed to 100, 91.2, 82.3, 76.7, 70.4, 60.7 and 49.0 per cent relative humidities by using sulphuric acid of different specific gravity (Leesage, 1895, Stevens, 1916). Maximum growth and sporulation were observed at 82.3 per cent relative humidity. The mycelial growth of the fungus was greater at this relative humidity on the fourth day and there was a further increase in growth on the fifth day, in other humidities further growth ceased on the fourth day.

Effect of hydrogen ion concentration on growth and sporulation. The organism was grown on Czapek's liquid and solid media and the pH adjusted at 2.1, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 levels. The fungus grew over a wide range of pH ranging from 3 to 9, but the maximum growth was observed at pH 5 in both liquid and solid media and no growth occurred at pH 2.1. Acervulus formation was much more in alkaline medium than in acidic medium.

Summary and Conclusions

The conidia of *Colletotrichum capsici* (Syd.) Butler and Bisby germinate readily in tap water within four hours. Spore germination is maximum at 30 °C. The percentage of spore germination increased with the increase of temperature up to 30°C. No germination takes place below 100 per cent atmospheric humidity. Light has no effect on spore germination . Maximum spore germination takes place in neutral solution, i.e. pH 7 and in 1 per cent sucrose solution. However, leaf extract and leaf decoction are the most suitable substrates for the growth of germ tubes.

Mycelial growth was most profuse in Richard's Medium, whereas sporulation was maximum in Czapek's medium, amongst synthetic media. Amongst non-synthetic media, potato dextrose agar was best, both for mycelial growth and sporulation. The size of conidia and setae was also maximum on this medium. The omission of either of the constituents of Czapek's medium affected both mycelial growth and sporulation.

The optimum temperature for the growth of the fungus in culture was around 30°C. No growth occurred at 7.2°C. and only slight growth occurred at 35.5°C. The optimum relative humidity for the growth of the fungus at 30°C. was found to be 82.5 per cent. Mycelial growth and sporulation were maximum in alternate light and darkness. The fungus grew over a wide range of pH, ranging from 3 to 9, the optimum lay around pH 5. No growth was observed at pH 2.1. Acervulus formation was much more in alkaline medium than in acidic medium.

Though the optimum temperatures for spore germination and the mycelial growth in culture were the same, there were differences in the requirements of light, humidity and pH of the medium in the two cases.

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OBSERVATIONS ON THE DOWNY MILDEW OF SETARIA VERTICILLATA BEAUV.

D. Suryanarayana and B. L. Chona

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Setaria verticillata Beauv., called *lapti*, *chirchira*, *bardani*, *chilaya*, and *chiklentha* in the Indian vernacular dialects, is a tropical or subtropical shade-loving grass, commonly found on the irrigation channel *bunds* in the villages in India. Blatter and McCann (1936) stated that the grass is eaten by the cattle in the young condition while the grain is consumed sometimes by the poorer classes.

During Novenber, 1956, in the Mycology Division area, we observed some of the adult plants of this grass with leaves almost dried up, producing pale yellow axillary shoots. They were conspicuous amongst the mass of dried up herbage of normal plants. In some of the leaves of these axillary shoots were found thick reddish brown oospores characteristic of the genus *Sclerospora*. The Sporangial stage of the fungus could not be observed at the time. However, on keeping these plants in a moist chamber after copiously spraying with water, the sporagiophores developed within 12 hours. Sporangiophores appeared as a downy white growth especially on the under surface of the leaves. Further observations could not be made during the season as only a few infected plants were available in the field.

In July, 1957, the patch of land where infection was seen during the previous season was kept under careful observation. After monsoon showers, large number of seedlings of this grass came up and some of them were found to be chlorotic. On the lower side of the leaves of such plants, typical downy white growth of sporangiophores was clearly visible. At this stage, the seedlings were 4 to 6 inches high. The infected seedlings remained stunted and produced a large number of tillers and a poorly developed root system (Plate I). At about this time, some of the healthy plants adjoining the infected ones showed chlorotic lesions 2 to 3 cm. long, often coalescing and covering appreciable area of the leaf surface (Plate II). Sporangiophores were also seen on the under surface of these lesions. Such infected plants dried up prematurely.

Histo-pathological studies of the infected plants showed typical Phycomycetous mycelium in the pith and cortex. (Plate III C). The hyphae were found to be both inter and intra-cellular. Haustoria were often seen and they were either simple or branched.

Sporangiophores emerged through the stomata in clusters of 2 or 3 and were characteristic of the fungus under study (Plate III A). They measured 217 to 288 μ in length. Occasionally, they showed basal cell

formation. Sometimes abnormal development of sporangiophores took place, particularly when the infected plants were kept in a saturated atmosphere for prolonged periods. Under these conditions, giant sporangiophores bearing larger sporangia were observed. The maximum length of such sporangiophores ranged up to 1024 μ while the sporangia on such structures measured 61 to 66 μ in length and 30 to 50 μ in breadth. In one case a young sporangiophore, which was still unbranched and club-shaped, was seen producing an outgrowth having a swollen end. Occassionally from the tip of a young sporangiophore- initial arose another hyphal growth. In another case, two side-branches of a sporangiophore developed into elongated structures, while the central branch bore normal sporangium. In still another case, the apices of sporangia were drawn into long beaks. Sometimes only branched mycelium was observed in place of sporangiophores as reported by Suryanarayana (1959) in the case of *Sclerospora* on *bajra*. Sporangia were round or oval and measured 18 x 14 μ. They germinated readily in water producing 3 to 5 zoospores which rounded up after sometime and germinated by germ tubes. Direct germination of sporangia was not observed (Figs. 1 to 9).

Oospores were seen in some of the distorted axillary shoots on the infected plants (Plate III B). They are pale yellow with smooth walls and have granular contents, and occupy the entire oogonial cavity but their wall is not united with the oogonial wall. Oogonial walls are thick, slightly convoluted on the outer side and reddish brown in colour. Oospores developed as usual in the parenchymatous tissue between the vascular bundles. Shredding of leaves after oospore formation, which is a common symptom in severals pecies of the genus *Sclerospora*, was not conspicuous in the present case. Oospores measured 19 to 29 μ in diameter while the diameter of the oogonial 'fruits' varied from 28 to 48 μ.

Green-ears consisting of small twisted leaves in the place of sound panicles were produced occasionally (Plate IV).

The two types of symptom pictures described by Wang (1936) in the case of *Setaria viridis* were clearly observed in the present case also. The first type is the outcome of systemic infection initiated in the seedling stage by the oospores of the previous season surviving in soil. This type of symptom picture is characterised by pronounced stunting and general chlorosis followeed by necrosis and green-ear formation. The other type is local infection caused by zoospores produced from the sporangia and it consists of chlorotic local lesions already described.

The causal fungus resembles morphologically the strain of *Slcerospora graminicola* (Sacc.) Schroet. described on *Setaria viridis*. It was first reported by Mitra (1941) from Allahabad on *S. verticillata* but he did not observe the oospore stage. He also did not differentiate the two types of symptom pictures so conspicuous in this case.

Acknowledgment. Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology and Joint Director,

Plate. I. A. Healthy plant
B. Infected plant showing pronounced stunting, tillering and poorly developed root system.

Plate. II. Linear local chlorotic lesions on the leaf surface caused by the zoospore infection.

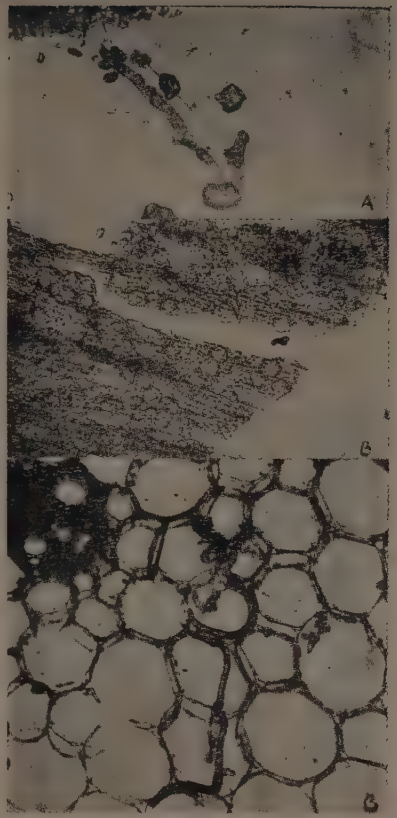

Plate. III. A. Sporangiophore with Sporangia attached.
B. Oospores embedded in the leaf tissue.
C. Mycelium in the pith of the stem of the affected plant showing inter and intra-cellular hypae.

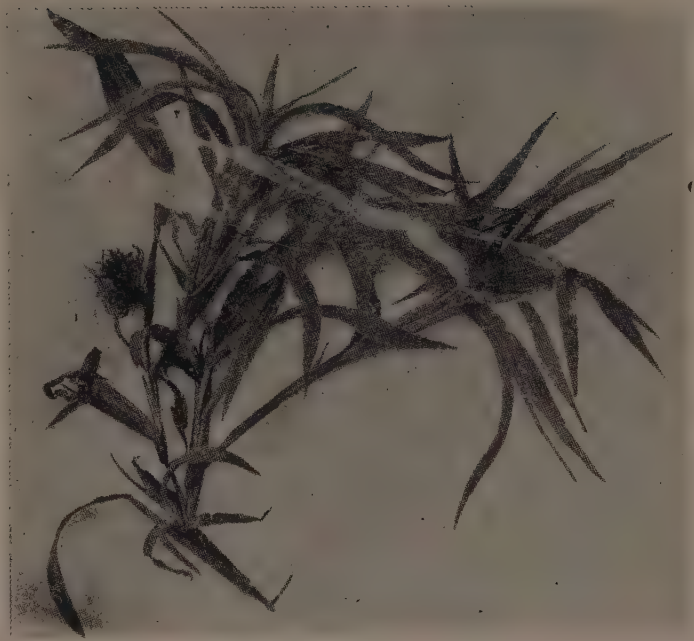

Plate. IV. An infected tiller showing a green-ear and a coiled axillary shoot below it.

Fig. I. A young sporangiophore-initial club-shaped and unbranched, (inverted)
2a The base of a fully mature sporangiophore with basal septum.
2b Top portion of sporangiophore showing branches bearing sporangia.
3. Basal part of a sporangiophore showing a basal septum.
4. Sporangia, one with zoospores.
4a Zoospores
4b Germinating zoospores.
5-9 Abnormal sporangiophores.

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Division of Mycology and Plant Pathology
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New Delhi.

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YELLOW MOSAIC OF MUNG (PHASEOLUS AUREUS L.)

T. K. Nariani

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During the summer season of 1955, *Phaseolus aureus* L., locally known as *Mung* grown in the Institute area was found to be heavily infected with a mosaic disease. The disease was characterised by the presence of bright yellow patches on the leaves interspersed with green areas. In several cases complete yellowing of the leaves was also noticed and the infected plants were stunted in growth. The incidence of the disease varied from 20-30 per cent. As the disease was suspected to be of virus origin, transmission experiments were undertaken under insect-proof conditions to study the causal virus and its host range. The results of such tests have been reported in this paper.

Symptomatology The first symptoms of the disease appear on the young leaves in the form of mild scattered yellow specks or spots. The next trifoliate leaf emerging from the growing apex shows irregular yellow and green patches alternating each other (Fig. I). The leaf size is generally not much affected but sometimes the green areas are slightly raised and the leaves show slight puckering and reduction in size. The size of yellow areas goes on increasing in the new growth and ultimately some of the apical leaves turn completely yellow. The diseased plants usually mature late and bear a very few flowers and pods. The size of the pods is reduced and more frequently immature and small sized seeds are obtained from the pods from diseased plants.

Transmission of the disease

Mechanical inoculation. Attempts were made to transmit the disease by juice inoculation by rubbing freshly extracted juice of mosaic affected leaves on the healthy young seedlings of *Mung* raised from seed in the insect-proof glass house using carborundum powder as an abrasive. The disease however, could not be transmitted in this manner although the test plants were kept under observation for over two months after inoculation.

Grafting. Young shoots collected from diseased plants from the field were grafted on healthy *Mung* plants in the glass house by the wedge method and covered with Bell jars to provide humid atmosphere. The disease was readily transmitted in the successful grafts and the symptoms appeared in the young axillary shoots below the grafted portion in 12-15 days. All the leaves on the new growth showed typical yellow and green areas characteristic of the disease.

Insect transmission. As white fly, *Bemisia tabaci* Gen., was found to be most predominant insect on the *Mung* crop and it has been reported to be the vector of similar diseases on *Phaseolus lunatus* L. and *Dolichos*

lablab (Capoor and Verma 1948, 1950), attempts were made to transmit the disease through the white fly. Batches of 15–20 insects were fed on diseased leaves for 24 hours in microcages before transferring them to young healthy seedlings of *Phaseolus aureus* in the insect-proof glass house. The insects were allowed to feed for 24 hours after which they were killed by spraying with Ekatox. Typical symptoms of the disease appeared after 12–16 days in the months of July and August (Fig. 2).

Host Range. Host range of the virus was studied by inoculating different plant species by feeding viruliferous white flies. A culture of white flies was raised on healthy tobacco plants in the Insectary to provide a regular supply of the insects for the experimental tests. The source of inoculum on which white flies were fed for picking up the virus was provided by an artificially infected plant of *Phaseolus aureus* showing typical disease symptoms. White flies fed for 24 hours on the diseased plant in microcages were transferred to healthy seedlings of different plant species tested. After 24 hours the insects were killed with Ekatox spray and the plants kept under observation. The plant species which did not show any visible symptoms after 1½ months after inoculation were indexed on *P. aureus* through white flies to determine if they were symptomless carriers. The results of the inoculation tests are presented in Table I.

Table. 1. The results of inoculation of different plants species by feeding viruliferous white flies.

Plant species	Number of plants inoculated	Number of plants infected
Phaseolus mungo (Urid).	11	8
Phaseolus acutifolius	11	8
Phaseolus aconitifolius (Moth)	9	3
Phaseolus lathyroides	6	3
Phaseolus lunatus	18	0
Phaseolus vulgaris	6	0
Phaseolus calcaratus	12	0
Phaseolus trilobus	14	0
Glycine max (Soyabean)	11	5
Dolichos biflorus	6	4
Dolichos lablab	11	0
Canavalia ensiformis	4	0
Vigna sinensis (Cowpea)	4	0
Abelmoschus esculentus (*Bhindi*)	6	0

The data presented in the Table I show that the virus is transmissible to *Phaseolus mungo*, *P. acutifolius*, *P. aconitifolius*, *P. lathyroides*, *Glycine max* and *Dolichos biflorus*, but not to *Phaseolus lunatus*, *P. vulgaris*, *P. calcaratus*, *P. trilobus*, *Dolichos lablab*, *Canavalia ensiformis*, *Vigna sinensis* and *Abelmoschus esculentus*. None of the remaining plant species proved to be symptomless carriers when indexed on *Phaseolus aureus*. The characteristic symptoms produced by the virus on different plant species are described below.

EXPLANATION OF PLATES

Fig. 1. Healthy and diseased leaves of *Phaseolus aureus* L.

Fig. 2. Transmission of the disease by white flies, *Bemisia tataci* Gen. to *P. aureus* and *P. mungo*. Right. *P. aureus*. Left. *P. mungo*.

Fig. 3. White fly transmission of the virus to *P. aconitifolius*.

Fig. 4. White fly transmission of the virus to *Dolichos biflorus*. Diseased leaves

Phaseolus mungo. The symptoms produced on this host were essentially similar to those on *Phaseolus aureus.* Bright yellow and green patches appear on the young leaves after about 12—15 days and continue to appear in the new growth (Fig. 2).

Phaseolus acutifolius. The first symptoms of the disease appear after 18–25 days after feeding viruliferous white flies and the plants show pale yellow chlorotic areas on the leaves. Unlike *Phaseolus aureus* and *P. mungo* the yellow areas are not irregularly scattered but are restricted to the interveinal areas and the veins and the midrib remain green. The size of the leaves is greatly affected and so also the internodes which are shortened and the growth of the plants is stunted.

Phaseolus aconitifolius. The leaves show faint pale yellow chlorotic pin points which increase in size and coalesce to form large yellow patches (Fig. 3). Very few large yellow patches are noticed and most of the leaves exhibit only small circular yellow spots which are irregularly scattered all over the leaf surface. There is no appreciable reduction in leaf size.

Phaseolus lathyroides. The leaves show chlorotic areas and severe malformation accompanied by raised blister like areas. The size of the leaves is greatly reduced. The green patches are darker than usual and the margins of the leaf lamina are irregular and wavy.

Glycine max. The symptoms appear after about 18—19 days after feeding viruliferous white flies. The young leaves show faint chlorotic areas on the leaves which turn yellow in due course of time. The yellow areas are more predominant in the new growth as in the case of *Phaseolus aureus* and *P. mungo* and increase in size to form large yellow patches.

Dolichos biflorus. The main symptoms on this host are faint chlorotic areas which remain light green and do not assume the yellow colour (Fig. 4). These chlorotic areas persist and the size of the leaflets is reduced. Some of the leaflets are slightly malformed and have irregular leaf lamina. The plant is greatly dwarfed due to reduction in leaf size.

Discussion. Two white fly transmitted 'yellow mosaic' diseases have been described on *Phaseolus lunatus* and *Dolichos lablab* respectively from Poona (Capoor and Varma 1948, 1950). Both these viruses differ in host range from each other and from the virus described herein. The virus under study is not transmissible to *P. lunatus* and *D. lablab.* Although the 'yellow mosaic' of *Phaseolus lunatus* is transmissible to *P. aureus,* it differs from the virus under study as it is not transmissble to *P. aconitifolius* but transmissible to *P. vulgaris* and *Canavalia ensiformis.* The host range of 'yellow mosaic' virus of *Dolichos lablab* has not been studied although it has not been found to be transmissible to *P. lunatus.* The present virus on *P. aureus* is, therefore, considered to be distinct from the above two viruses. It is proposed to name the virus under study as "Yellow mosaic" of *P. aureus.*

Summary

A yellow mosaic disease of Mung (*P. aureus*) has been described. The disease is characterised by the presence of bright yellow patches on the leaves interspersed with green areas.

The disease is graft transmissible but is not transmitted by mechanical means. White fly, *Bemisia tabaci* Gen. transmitted the disease to *Phaseolus aureus*, *P. mungo*, *P. acutifolius*, *P. aconitifolius*, *P. lathyroides*, *Glycine max* and *D. biflorus* but not to *P. lunatus*, *P. vulgaris*, *P. calcaratus*, *P. trilobus*, *Dolichos lablab*, *Canavalia ensiformis*, *Vigna sinensis* and *Abelmoschus esculentus*.

The virus differs from the "yellow mosaic" diseases described on *P. lunatus* and *D. lablab* in host range and its inability to infect these plant species.

Acknowledgment. Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest and helpful suggestions throughout the course of these investigations.

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VARIATION IN THE GUAVA WILT PATHOGEN, FUSARIUM OXYSPORUM f. PSIDII

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In Uttar Pradesh, India, guava (*Psidium guajava* L.) is one of the economically important fruit crops. Among the diseases affecting the culture of this crop, the wilt disease which was first observed in 1935 in the district of Allahabad, is the most destructive. The disease is rapidly spreading and in 1952 it is reported to have spread to nearly 20,000 square miles in Uttar Pradesh. A *Fusarium* sp. was found by Das Gupta and Rai (1947) to be the causal agent of the disease. The specific epithet *Fusarium oxysporum* f. *psidii* was proposed for the fungus by Prasad *et al* (1952). The symptoms of the disease are typical of any vascular wilt caused by *F. oxysporum.*

The present paper includes work on pathogenicity and morphological and cultural characters of ten isolates of this fungus.

Material and Methods. In the present work ten isolates of *Fusarium* spp. (F_1 - F_{10}) were used. The isolate F_1 was *Fusarium oxysporum* f. *psidii* kindly sent by Dr. R. S. Mathur, Government Plant Pathologist, Kanpur, U. P., India, whereas the remaining isolates, F_2 - F_{10} were single spore cultures of *Fusarium* made by the author from roots of wilting trees of guava in and around the Allahabad Agricultural Institute Campus.

The cultures of the *Fusarium* isolates grown on sterilized oat kernels for 20 days served as inoculum. This was incorporated into sterilized soil contained in steam sterilized pots and incubated for three days before planting the seedlings. For successful infection 24 g. of inoculum for every 500 g. of soil was used (Fig.1).

Two varieties of guava viz., "Safeda" and "Lalguda" were used. The former is a choice table variety raised in Allahabad whereas the latter, being hardy, is used as a root stock in some nurseries. Seeds of these varieties were obtained from India. Surface sterilized seeds were planted in steam sterilized soil to raise seedlings. For infection tests the seedlings were uprooted and the roots washed free of soil under running tap water taking care to avoid root injury. Later they were transplanted into the the inoculated potted soil. In controls sterilized oat kernels were used in the place of inoculum.

The plants were kept in a green house where the temperature varied between 85° –100°F. The pots were watered with tap water as and when required. Seeds were surface disinfected before planting and roots prior to plating by shaking in 5% sodium hypochlorite for 1–2 minutes.

EXPERIMENTAL. The *Fusarium* isolates, F_1–F_{10}, were tested for pathogenicity on guava seedlings. In the first test 50-day-old seedlings of two varieties of guava, "Safeda" and Lalguda" were planted in soil inoculated with the 10 isolates of *Fusarium*. Each treatment was replicated three times with 2 plants per pot. The second test was a repetition of the first test except that 30-day-old seedlings of "Safeda" variety only were used, with 5 seedlings per pot. In the third test reisolates of *Fusarium* that proved to be most pathogenic in the second test were tried for pathogenicity on 160-day-old seedlings of "Safeda". Each treatment was replicated 4 times with 5 plants per pot. The results of these tests are furnished in Tables 1–3.

TABLE. 1. Pathogenicity trial with 10 isolates of *Fusarium* on two varieties of guava

	Isolates										
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	Check
L % *	100	100	33	0	0	0	67	100	50	17	0
S death	83	83	33	0	0	0	83	100	50	0	0

* Percentage based on 6 plants
L — "Lalguda"
S — "Safeda"

TABLE. 2. Pathogenicity trial with 10 isolates of *Fusarium* against "Safeda" seedlings.

	Isolates										
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	Check
% * death	67	80	27	0	0	0	47	80	40	0	0

* Percentage based on 15 seedlings.

TABLE. 3. Pathogenicity test with 5 reisolates indicated to be most pathogenic in test 2, on "Safeda" seedlings.

Reisolate	No. Dead	% Death *
F_1	16	80
F_2	17	85
F_3	13	65
F_8	17	85
F_9	18	90
Check	1	5

* Percentage based on 20 seedlings

Cultural and morphological characters of the isolates of *Fusarium* were studied to determine whether all the isolates belonged to one or more

species of *Fusarium*, and also whether there was correlation between their cultural and morphological characters, and pathogenicity. The isolates were grown in potato-dextrose agar and malt-agar media for a period of 8 days at 30°C. to record radial rate of growth. The rate of growth was faster in the potato-dextrose agar with deeper pigment production. There was no marked difference in rate of growth among isolates on any one medium. The range in diameter among isolates in potato-dextrose agar was 8.3–9.0 cm. whereas in malt agar it was 7.3-8.0 cm. Based on the pigment diffused into the medium the isolates could roughly be divided into three groups. The isolates F_1, F_2, F_3, and F_4, produced "drab" colour whereas F_5, F_6, F_7, F_8 and F_9 produced "vinaceous russet" and F_{10} "dark vinaceous purple" (Ridgway 1912).

To stimulate macroconidial production all the isolates were kept in darkness for a week and later exposed to light for three weeks (Harter, 1941) The isolates F_1, F_2, F_3, and F_4 developed macroconidia in sporodochia and pionottes and they were typical of the *Elegans* section of Wollenweber and Reinking's (1935) system of classification (Fig. 2). The isolate F_9 produced macroconidia in sporodochia, rather sparingly. Although the the macroconidia were typical of *Elegans* section, they were somewhat slender and less curved than those of F_1, F_2, F_3, and F_4, and the size of conidia were within those given for *F. oxysporum* (Fig. 3). The isolates F_5, F_6, F_7, F_8 and F_{10} failed to produce any macroconidia during the period of study and these varied widely in their pathogenicity. In the absence of macroconidial production it would be difficult to classify these isolates. However, these could be considered strains that had either lost their ability to develop macroconidia or might produce them under special conditions. Most of the important characters of the isolates mentioned above are summarized in Table 4.

Isolates F_1, F_2, F_3, F_8 and F_9 could be classed as highly pathogenic while the remaining isolates caused little or no disease under the conditions tested. Although all the isolates belong to *F. oxysporum* f. *psidii*, for reasons substantiated elsewhere, their cultural characters are not similar. No correlation was found betwwen the cultural characters and pathogenicity.

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Summary.

Fusarium oxysporum f. *psidii*, Prasad, Mehta, and Lal exists in a variety of clonal forms that differ in pathogenicity, and in morphological and cultural characters. No correlation was observed between the cultural characters and pathogenicity.

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Fig. 1. Left—Healthy seedlings in soil without the pathogen
Right—Wilted seedlings in infested soil.

Fig. 2. Macroconidia produced by the *Fusarium* isolates, F_1, F_2, F_3, and F_4.

Fig. 3. Macroconidia produced by the *Fusarium* isolate, F^9

TABLE. 4. Morphological and cultural characters of the ten isolates grown in potato-dextrose agar medium*

Isolates	Radial growth at 30 C 8 days (mm)	Colour diffused into medium	Aerial mycelium, colour and growth	Sporodochia and pionnotes	3-septate macroconidial measurements(μ)
F_1	90	drab	dull white; fair	present	25.2 - 33.4 x 2.8 - 4.2
F_2	86	drab	dull white, fair	present	28.0 - 34.8 x 2.8 - 4.2
F_3	88	drab	dull white, good	rarely present	23.8 - 35.2 x 2.8 - 4.2
F_4	90	drab	dull white, fair	present	25.2 - 35.2 x 2.8 - 4.2
F_5	89	vinaceous russet	white with light pink tinge, abundant	not observed	
F_6	90	vinaceous russet	white with pink tinge, abundant	not observed	
F_7	85	vinaceous russet	white with pink tinge, good	not observed	
F_8	90	vinaceous russet	white with pink tinge, abundant	not observed	
F_9	83	dark vinaceous purple	pink tinge, good	only sporodochia present	26.6 -42.0 x 2.8 - 3.8
F_{10}	85	dark vinaceous purple	pink tinge abundant	not observed	

*In all isolates microconidia, and intercalary and terminal chlamydospores were observed.

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"GRASSY-SHOOT" DISEASE OF SUGARCANE

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Introduction. A serious disease of sugarcane, locally known as "grassy-shoot", was observed and collected in 1949 from the Maharastra Sugar Mills near Belapur in the Ahmadnagar District of Bombay (Chona, 1958). Investigations of the disease were begun in 1951 but it was first recorded in 1955 (Vasudeva, 1955). The disease is now widsepread in the Deccan Canal Tract of the Bombay State (Arkeri and Patel, 1955) 1955), and threatens the cultivation of the most productive and prized Co. 419 variety of sugarcane (Albuquerque and Arkeri, 1957) although it was also observed to attack other cane varieties in nature. Symptoms of "grassy-shoot" have also been observed in a few cases of the nucleus seed-cane material received in recent years at some stations from the Sugarcane Breeding Institute, Coimbatore. The disease has also been reported from Andhra Pradesh, Bihar, Uttar Pradesh, and Madras. In the Bombay Deccan it is fairly widespread and has been observed to occur at Baramati, Malegaon Sugar Factory, Padegaon Sugarcane Research Station, and Poona in the Poona District; at Belapur Sugar Factory (Harigaon), and Changdeo Sugar Factory (Puntamba) in the Ahmadnagar District; and at the Ravalgaon Sugar Farm Ltd., in the Nasik District. In most places the incidence of the disease in the plant crop (*Adsali* crop) is sporadic, but is almost cent per cent in the ratoon crop which is most severely affected. The observations made and the investigations carried out with a view to identifying the disease, and establishing its mode of transmission, the insect vectors, collateral hosts, and other characteristics are recorded here.

Material and Methods. Stools of "grassy-shoot" affected Co. 419 sugarcane were grown in 18-inch pots in insect-proof glasshouses and material propagated from setts of affected canes were used as source of the disease in all the experiments carried out. Diseased material was also obtained as and when required from the Maharastra Sugar Mills and also from the Walchandnagar Sugar Factory through the courtesy of their managements. Healthy plants for all the experiments were raised inside the glass-houses from setts of Co. 419 obtained from a source known to be free of "grassy-shoot". Adequate control plants were maintained for each experiment.

The aphid colonies were raised on healthy sugarcane in the insectary and populations taken from these were used for the insect transmission tests except in a few preliminary trials when the insects were collected from diseased sugarcane in fields. Whether collected from outside or taken from the colonies in the insectary, the insects were first fed on diseased sugarcane plants for at least 24 hours before liberating them on healthy test plants. As a rule all plants fed upon by insects were sprayed with

0.02% solution of Folidol-E 605 before keeping them in a separate glass-house for observation.

Symptomatology. The "grassy-shoot" disease in nature is characterised by the production of numerous thin tillers from the base of the affected stools (Plate I. fig. 1). This profuse and stimulated growth gives rise to a dense or crowded bunch of tillers bearing pale yellow or chlorotic leaves which remain thin, narrow, and much reduced in size (Plate II, fig. 1). Each stalk that is produced from the affected stool shows a marked reduction of the internodes so that the nodes scars are closely set together, and, as each bud sprouts in turn giving rise to a lateral tiller, the resulting growth assumes the characteristic bushy appearance. On account of the continuous formation of thin tillers cane formation does not take place and the crop, therefore, suffers a serious economic loss.

The effect of the disease is far more severe when sugarcane is infected in the early stages of growth, or when setts for planting are obtained from apparently healthy but primarily infected cane (Primary Infection). The disease, however, shows up in the most severe form in the ratoon crop in which the clusters of slender tillers with reduced leaves usually growing erect give an appearance of a field full of perennial grass (Plate II, fig. 3), and from which it has derived its popular name: "grassy-shoot".

Infection of the "grassy-shoot" disease in the plant crop in advanced stages of growth shows up either in the form of pale-green or chlorotic leaves developing at the crown, or by the formation of numerous tillers from the base of the stool bearing pale-green leaves, or even by the development of both these symptoms on the same stool. The buds on affected and grown-up canes are usually scaly, abnormally elongated, and delicate, and as much as 50% loss in germination of such buds has been recorded. It has also been observed that the disease often induces premature sprouting of the scaly eye-buds which are stimulated to grow into slender and chlorotic shoots all over diseased canes (Plate I, figs. 1 & 2).

Premature sprouting of buds on sugarcane as induced by the "grassy-shoot" disease is of a fairly common occurrence although abnormal sprouting of buds on otherwise healthy sugarcane has also been observed in nature. Such cases were carefully investigated and it was conclusively shown that the premature sprouting of buds in the case of healthy canes was induced only when the growing shoot had been destroyed either by borers or by the 'twisted top' disorder, or even when the top was cut away accidentally. In all these cases it was observed that the buds close to the apex grew to replace the lost top-shoot and to continue the growth forward. Setts obtained from such canes and grown in a glasshouse always grew into normal plants. In other words, the abnormal sprouting of buds in such cases is only physiological in character and is not brought about as a result of infection of the plant by any disease. Abnormal features, such as "clustered shoot", "Witch's broom", or "Bunch top", the symptom picture of which could be mistaken for one of the many diseases of sugarcane, are also reported by Buzacott (1953).

Experimental

Transmission of the "grassy-shoot" disease:

Transmission in seed setts. A large number of setts were obtained from the ratoon as well as from plant crops exhibiting early as well as advanced stages of the disease and planted in pots to see the extent of disease transmission. Setts were also obtained from canes showing premature sprouting of nodal eye-buds and planted as above. These tests showed that majority of the setts produced plants showing typical "grassy-shoot" disease, while, as the data in Table 1 show, setts obtained from healthy canes produced only healthy plants. It was also observed that germination of buds on diseased setts was poor and the tendency to produce tillers became evident soon after the buds had germinated and started growing, unlike those growing from healthy setts which produced only single and stout shoots (Plate II, fig. 2).

Table 1. Transmission of "grassy-shoot" disease in setts obtained from affected sugarcane variety Co. 419

Date of planting	Source of setts	Number of setts		
		planted	germinated	diseased
November, 1953	Ratoon crop	15	10	10
November, 1953	Healthy cane (control)	10	10	10
March 1954	Ratoon crop	12	10	6
July, 1954	Ratoon crop	40	34	22
February, 1955	*Adsali* cane with sprouted nodal buds	28	26	26
February, 1955	Ratoon crop	28	22	22
March, 1955	Healthy cane (control)	31	31	0
June, 1955	*Adsali* crop	22	22	22
June, 1955	Ratoon crop	18	18	18
June, 1955	Healthy cane (control)	44	44	0
August 1956	*Adsali* cane with sprouted nodal buds	35	20	20
August, 1956	Healthy cane (control)	12	11	0
April, 1957	*Adsali* crop	8	6	6

Transmission by means of the cutting knife. In order to test the possibility of the disease being carried to healthy canes through the medium of contaminated cutting knife during the seed-cutting operations in the field, and thus spreading the infection to a large number of plants in the following crop, healthy Co. 419 canes were cut into single-budded setts with a knife that had been used to cut diseased canes, and these were then planted in pots inside the glasshouse and kept under observation for disease development. The disease, as the data in Table 2 show, infected 16 of the 36 setts that germinated out of a total of 81 setts that were planted. The results indicate that the disease easily spreads through the medium of the cutting knife in commercial plantations.

TABLE 2. Transmission of the "grassy-shoot" disease to healthy setts through the medium of the cane-cutting knife

Date of cutting	Number of setts		
	planted	germinated	diseased
15th April, 1954	20	10	2
15th April, 1954	10 (Healthy)	10	0
7th August, 1956	22	7	4
10th August, 1956	15	0	0
5th April, 1957	24	19	10
,, ,, ,,	8 (Healthy)	5	0

TRANSMISSION BY MECHANICAL MEANS. In order to see if the "grassy-shoot" disease was transmitted through juice inoculation, preliminary tests were carried out with juice extracted from chlorotic leaves of diseased plants and inoculated with it about 9 inches high healthy plants of Co. 419 sugarcane. The inoculations were made both by leaf-rubbing with the help of carborundum powder and also by pin-pricking through the inoculum into the basal portion of the spindle of 32 healthy plants. The disease was not transmitted in any of the inoculated plants.

In subsequent trials, juice extracted from chlorotic leaves and also from stalks of diseased plants by crushing these separately in a 0.5% solution of Na_2SO_3 was used to inoculate single-eye setts of healthy Co. 419 cane. These were inoculated repeatedly by pricking the cut-ends after dipping them in the inoculum. Similarly each sett was also inoculated in the region of the root primordia on the node. Half an hour after inoculation the setts were planted individually in 8-inch pots and kept for observation. The disease was readily transmitted to canes inoculated with juice extracted from diseased leaves (Plate II. fig. 4), but, as is clear from Table 3, it was not transmitted into setts which were inoculated with juice extracted from diseased stalks.

TABLE 3. Transmission of the "grassy-shoot" disease by juice inoculation of setts of Co. 419 cane

Date of experiment	Source of inoculum	Number of setts		
		inoculated & planted	germinated	infected
7th August, 1956	Diseased stalks	7	7	0
9th August, 1956	Chlorotic leaves	15	10	8
4th May, 1957	Chlorotic leaves	26	22	18

This method of inoculation proved very successful and was adopted for all the future inoculation tests. The only disadvantage was that the symptoms of disease developed after 5 to 8 months and distinct "grassy-

shoot" symptoms showed up about 4 to 6 months later. This is believed to have been brought about on account of the fact that in all setts, whether inoculated or not, a single primary shoot was produced on germination of the bud, and it usually took a long time before the secondary shoots were thrown out by the plant under glass-house conditions. This difficulty was overcome by cutting away with a sterilised knife the primary shoot near the base after it had grown for about 3 months. This operation induced the plant to put forth new shoots earlier than usual. Such new shoots in the case of infected plants were numerous, slender, and often chlorotic; but in the case of the controls usually a few stout shoots bearing broad dark-green leaves were produced. Thus, when a sett had contracted the disease following mechanical inoculation, typical "grassy-shoot" symptoms appeared soon after the secondary buds started growing from the planted setts.

Transmission by insects. Attempts at insect transmission of the disease were made through a blackish-brown aphid found colonising on sugarcane at Poona, the palish-green sugarcane aphid (Vasudeva, 1956), and the corn aphid. The black-green aphid species has been identified as *Aphis idiosacchari*, nov. sp., in order to distinguish it from the palish-green sugarcane aphid *Aphis sacchari* Zehntner (*Longiunguis sacchari* Zehntner) which is a very common pest of sugarcane. It also breeds abundantly on Jowar (*Sorghum vulgare* Pers.). The blackish-brown *Aphis idiosacchari*, however, has not been observed to breed on Jowar. The corn aphid, *Aphis maidis* Fitch,. was collected in very small numbers from sugarcane, but plentifully from corn and Jowar. This is a vagrant species and it has been found to breed on several grasses as well as on corn (*Zea mays* L.), besides sugarcane and Jowar.

In addition to the aphid species the following insects were also observed to breed on sugarcane in nature in the vicinity of Poona and at Walchandnagar:

Saccharicoccus sacchari (Cockerell), mealy bugs.

Aleurolobus barodensis Msk., white fly, and

Pyrilla perpusella Walk., fulgorid.

Except the fulgorid, all other insects were used in transmission tests of the "grassy-shoot" disease. The results of these tests, given in Table 4, show that the disease was readily transmitted by *Aphis idiosacchari*, *Longiunguis sacchari*, and *Aphis maidis*, showing thereby that the "grassy-shoot" disease is an aphid-borne virus. The data also show that *Longiunguis sacchari* is the most efficient vector of the virus, while *Aphis idiosacchari* and *A. maidis* rank next in order of efficiency in transmission of the disease. The mealy bugs and the white-flies failed to transmit the disease.

Collateral Host of "Grassy-shoot" Virus. Jowar (*Sorghum vulgare*) growing near the affected sugarcane fields at Walchandnagar was observed to show stunted growth with leaves turning partly or completely

chlorotic. Such plants also had numerous chlorotic and slender shoots arising from below the soil-level and very much resembling the "grassy-shoot" disease of sugarcane. Similarly affected Jowar plants were also observed at Poona and other places nearby. These observations indicated that the disease in Jowar may be identical with the one that affects sugarcane. Tests were, therefore, carried out to see if the disease in sugarcane and Jowar is caused by the same virus.

TABLE 4. Insect transmission of "grassy-shoot" disease of sugarcane

Date of experiment	Insect species	Number of insects per plants	Number of plants colonised	infected
July. 1955	*Aphis idiosacchari*	15 to 20 mixed	7	4
March to April, 1957	,, ,,	30 to 50 mixed	38	24
July, 1955	*Longiunguis sacchari*	30 mixed	6	3
Oct., 1955	,, ,,	50 apterous	4	4
Nov., 1955 to July, 1956	,, ,,	—	13	5
	,, ,,	—	20	3
Jan., 1956	,, ,,	20 alate & 20 apterous	6	4
Aug., 1956	,, ,,	50 apterous		
		15 alate	4	4
Sept., 1956	,, ,,	50 alate	4	4
Oct., 1956	,, ,,	50 to 60 apterous	5	3
Nov., 1956	,, ,,	20 alate & 30 apterous	1	1
Dec., 1956	,, ,,	50 to 60 apterous	20	17
Jan., 1957	,, ,,	30 alate	6	4
Sept., 1955	*Aphis maidis*	50 apterous	2	0
Nov., 1955	,, ,,	20 alate & 30 apterous	10	6
Feb., 1956	,, ,,	50 apterous	4	2
March to April, 1957	,, ,,	50 mixed	26	21
Oct., 1955 to March, 1956	*Saccharicoccus sacchari*	30 to 50 females	23	0
April, 1956 to June, 1956	*Aleurolobus barodensis.*	50 adults	14	0

TRANSMISSION OF JOWAR "GRASSY-SHOOT" TO JOWAR AND SUGARCANE THROUGH APHID. For these tests *Longiunguis sacchari* was colonised on diseased plants of Jowar collected from the field. Insects from this colony were transferred to healthy seedlings of Jowar grown from seed, and also on healthy plants of Co. 419 cane in batches of 30 to 50 adults per plant. Of the 15 seedlings of Jowar and 8 plants of Co. 419 cane which were ino-

culated, 11 Jowar seedlings and 3 cane plants developed typical "grassy-shoot" disease, indicating that the Jowar virus produced typical "Grassy-shoot" disease in sugarcane.

Transmission of Sugarcane "Grassy-shoot" to Jowar through the sugarcane aphid. Apterous as well as alate viviparous females of *Longiunguis sacchari* were fed on diseased sugarcane for 16 days and then transferred in batches of 30 to 50 insects on each of the 15 healthy seedlings of Jowar. Of these 14 Jowar plants developed typical "grassy-shoot" disease while none of the controls on which non-viruliferous aphids were liberated were infected. This experiment confirmed that the Jowar "grassy-shoot" is caused by the same virus that produces the "grassy-shoot" disease in sugarcane.

These observations were further confirmed by successfully transmitting the virus from Jowar plants experimentally infected with the sugarcane "grassy-shoot" virus to healthy seedlings of Jowar and also to Co. 419 cane plants. Similarly, 24 young healthy Co. 419 plants and 15 Jowar seedlings were inoculated by means of *Longiunguis sacchari* taken from colonies reared on the "grassy-shoot" affected Jowar plants. Of these 22 cane plants and 14 Jowar seedlings developed typical "grassy-shoot" symptoms.

Discussion. The observations made on the "grassy-shoot" disease of sugarcane prevalent in the Deccan Canal area of the Bombay State and the experimental data presented herein conclusively show that the disease is caused by a virus which in nature is transmitted by at least three species of aphids, i.e., *Longiunguis sacchari*, *Aphis idiosacchari*, and *A. maidis*. The disease is also readily transmitted through setts obtained from diseased canes, through the medium of the cane-cutting knife, and by juice inoculation into the cut ends of healthy canes. The disease, however, was not transmitted to young cane plants by pin-pricking through infective leaf extract. Extract of leaves ground to a 0.5% Na_2SO_3 solution was found to be very infective, but juice extracted from diseased stalks did not produce any disease in tests reported herein.

Jowar (*Sorghum vulgare*) which in nature is also affected by a similar disease, has been shown to be a collateral host of the sugarcane "grassy-shoot" virus. The disease appears to perpetuate in Jowar even in fields far away from sugarcane cultivation. Since the aphid vectors, except *Aphis idiosacchari*, breed abundantly on Jowar, this plant acts as a potential reservoir of the virus which is probably carried to sugarcane when the aphids migrate from Jowar to sugarcane. Although Hughes (1955) reported that the ratoon-stunting disease of sugarcane infects corn and sweet sorghum, from which the disease could readily be transferred to sugarcane, he did not observe the disease affecting these crops in nature. This is probably due to the fact that no effective insect vector of the ratoon-stunting disease has yet been discovered.

The "grassy-shoot" disease reported herein does not resemble the ratoon-stunting disease of sugarcane prevalent in Queensland, Australia

(Steindl, 1950; Steindl & Hughes, 1953) and also recorded from other countries (Hughes and Steindl, 1955), and differs from it principally in respect of its symptom picture, in being aphid-borne, and in its effect on the plant crop as well. The prominent symptoms caused by the "grassy-shoot" virus are distinct chlorosis of leaves of the affected canes, excessive tillering and absence of cane formation (Plate II, fig. 3) but the type of stunting of the ratoon crop as is found in the case of sugarcane affected by the ratoon-stunting disease is not produced. Even the grown-up plant crop develops chlorotic leaves at the crown and shows premature sprouting of the lateral buds as a result of primary infection by the "grassy-shoot" virus, and occasionally the affected stool though appearing healthy puts up slender chlorotic shoots from the ground level. The diseased plants in a maturing plant crop stand out prominently on account of these symptoms.

The "grassy-shoot" disease of sugarcane is, therefore, a new virus disease of sugarcane not recorded previously.

Summary

A serious disease of sugarcane designated as "grassy-shoot" has been described.

The disease is transmitted through infected seed setts, through cane-cutting knife and by mechanical means through juice of diseased leaves.

Longiunguis sacchari Zehntner, *Aphis idiosacchari* nov. sp., and *A. maidis* Fitch, have been established to be the vectors of the virus.

Jowar, *Sorghum vulgare* Pers. has been shown to be a collateral host of the virus.

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EXPLANATION OF PLATES

PLATE–I

Fig. 1. "Grassy-shoot" affected stools of sugarcane variety Co. 419 showing numerous thin tillers arising from the base and having chlorotic leaves. Note premature sprouting of eye-buds on the single thicker shoot.

Fig. 2. A mature Co. 419 cane affected by "grassy-shoot" showing premature sprouting of nodal eye-buds into slender chlorotic shoots.

PLATE–II

Fig. 1. "Grassy-shoot" infected Co. 419 sugarcane stool showing continuous tillering and absence of cane formation. Stool collected in 1949 and grown in pot. Photographed in 1957.

Fig. 2. Healthy (right) and "grassy-shoot" affected Co. 419 plants (left) raised from healthy and diseased setts, respectively.

Fig. 3. A "grassy-shoot" affected ratoon crop of Co. 419 sugarcane. Note chlorotic leaves on tillers and absence of cane formation.

Fig. 4. Co. 419 cane plants grown from single-budded setts and infected by juice inoculation into the cut ends. Healthy plants on extreme right.

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MUTATION IN COLLETOTRICHUM FALCATUM WENT, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE III. SOME BIOLOGICAL EFFECTS OF METHYL BIS (B-CHLOROETHYL) AMINE

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Introductory: Artificial mutagens, e.g., chemicals, radiations and radiomimetic agents like nitrogen mustards and sulphur mustards have been effectively used during the recent years for inducing variation in micro-organisms. It is generally believed that a highly reactive unsaturated nitrogen atom of the mustard gas reacts with various groupings of nucleoproteins which are concerned in transmission of hereditary characters and that this activity is further enhanced by the type of side chains present in the vesicant mustard (Foster, 1949). Methyl Bis (β-chloroethyl) amine (MBA) or nitrogen mustard has been used during the last decade or so for inducing variations in *Penicillium notatum* (Stahman and Stauffer, 1947), *P. chrysogenum* strain Q. 176 (Reese *et al.*, 1949), *Neurospora* (McElroy *et al.*, 1947, Tatum *et al.* 1950), *Phoma lingam* (Calvert *et al.*, 1949) and some other organisms (Tatum and Perkins, 1950).

Variation in red rot organism of sugarcane, particularly in pathogenicity, is of common occurrence and it has been responsible for repeated epidemics of the disease in sugarcane crop. In order to determine the factors that influence such a change and to know about the nature of variability in this organism a research project on this aspect which also includes a study of induced mutation with the aid of artificial mutagens has been taken up (Vasudeva *et al.*, 1957, 1958; Bajaj *et al.*, 1959) and the biological effects of nitrogen mustard on *Colletotrichum falcatum* Went. are reported in this paper.

Experimental: A heavy spore suspension of a stable light type isolate No. 244 of *C. falcatum* obtained from the Indian Type Culture Collection was prepared in sterile distilled water from 10-day-old culture and the concentration adjusted to nearly 15 million spores per ml. as determined by Haemacytometer.

A stock solution of 0.1 mM. concentration of nitrogen mustard was prepared by diluting Di (2-chloroethyl amine) methyl amine hydrochloride (Mustine hydrochloride) of Boots Pure Drug Company, Ltd., England (10 mg. vial) with double distilled water by means of hypodermic syringe. The entire procedure was carried out under aseptic conditions and necessary precautions were taken as the chemical is a powerful vesicant. The method of treatment of spores was somewhat similar to that of Stahmann and Stauffer (*l.c.*); 0.25 ml. of the stock solution was diluted 10 times with 3.2 mM. $NaHCo_3$ and after a small interval, 10 ml. of the spore suspension

of the fungus was added to it and allowed to stand at room temperature 27 C. The final concentration of MBA at the time of treatment was 0.00125 mM.

At intervals of 1, 2, 4, 8, 16, 32, 64 and 128 minutes, 0.4 ml. aliquots were withdrawn and added to 50 ml. of sterile decontaminating solution containing 60 mg. (0.8 mM) of glycine and 68 mg. (0.8 mM) of sodium bicarbonate. The control was maintained by adding 10 ml. of standard spore suspension to an equal volume of 3.2 mM $NaHCO_3$ and after 128 minutes 0.4 ml. of the suspension was withdrawn and added to 50 ml. of decontaminating solution. The samples of treated as well as untreated spores were shaken thoroughly in decontaminating solution and stored at 4°C. for 24 hours or longer.

The experiment was repeated twice with 0.01 mM and 0.1 mM final concentrations of MBA and the period of treatment raised to 24 hours. The remaining procedure was almost the same as discussed above.

The spores after treatment were washed by centrifugation and were inoculated on oatmeal agar medium in the centre of the petri-plates for observations on their growth. The inoculated cultures were incubated at room temperature and final observations recorded after 20 days. The comparative rate of germination of the treated as well as untreated spores was also studied by the usual slide method in the treatment with only highest concentration of MBA.

The growth rate studies of the variants as well as the parent culture were carried out on oatmeal agar and Dextrose-asparagin—thiamine media (Dextrose 30 gm., asparagin 1 gm., $MgSO_4 . 7H_2O$ 0.5 gm., KH_2PO_4 1.5 gm., thiamine 10 mg., distilled water to make up 1 litre) in petri-plates of 10 cm. diameter. Plates were inoculated with spore suspension in 4% plain agar, standardized at an equal level in each treatment. The plates were incubated at room temperature (20.5° to 30°C.) and the observations were recorded every third day by measuring the diameter of the colonies at three different angles and the average of the three readings was taken as the final value of the colony.

The size of the conidia of different variants was determined by recording measurements of 200 spores of each after 15 days' growth on oatmeal agar at room temperature (20.5° to 30°C). The germination of the conidia of the variants of the same age was studied on 0.02% yeast extract by the slide method. The observations were recorded after 24 hours of incubation at 21°–22°C under high humid conditions.

The observations on the germination of the spores of *C. falcatum* treated with 0.1 mM of BMA (Fig. 1) showed that the germination of the treated spores was lower than that of the untreated ones. It was further depressed as the time of treatment was increased,—only 20% in 24 hours treatment as against 70% in the control.

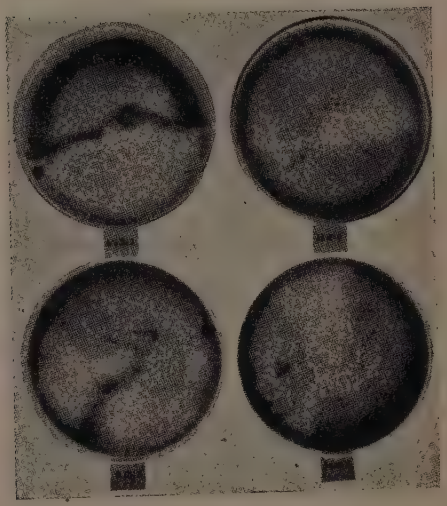

Fig. 1.

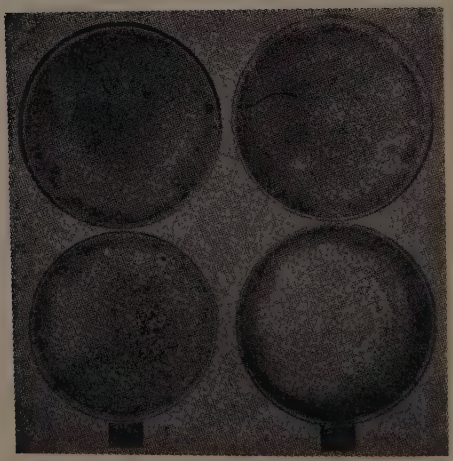

Fig. 2.

The observations as recorded after 20 days in the lowest treatment (0.00125 mM) of MBA showed variation only in the form of scattered mycelial patches on the surface of the medium which on subculturing on oat agar slants resulted into typical dark type* cultures of *C. falcatum*. The remaining part of the petriplates showed normal growth, characteristic of the parent without any indication of sector formation. The treatment of spores with 0.01 mM MBA resulted in distinct sector formation in majority of the plates. The sectors were generally of regular shape resembling "V, Wedge", and 'Fan" shapes though irregular sporulating patches were also observed. The size of the sectors also varied from plate to plate. The sporulation in the sector portion was generally slimy and masses comparatively thinner than those of the parent. In addition, variations in the form of mycelial patches as also in the intensity and nature of sporulation were also observed. The control plates showed normal growth and sporulation. The experiment was conducted on small scale and it was difficult to measure the rate of variation corresponding to the dose of MBA but some idea about the frequency of variation could be had as majority of the petri-plates showed distinct sectoring. The period of treatment, however, had no marked effect on the frequency of sector formation.

Subscultures were made from different portions of these sectors on oat-meal agar which resulted in about 8 cultures differing from each other in morphological characters. These eight variants could be broadly grouped in two categories; the first having profuse sporulation with comparatively slimy and thinner spore masses and silky mycelial growth and the second being somewhat similar to the well known dark type with chlamydospores and profuse, grey and cottony aerial mycelium. There were no apparent pink spore masses in the latter but sparse sporulation could be observed under the microscope, particularly on the surface of the medium covered with cottony mycelium. Single spore cultures of the two representative types and the parent were established and designated as C_1, C_2 and C, respectively, for further studies.

The treatment with 0.1 mM of MBA resulted in sector formation in almost all the cases and had a wide range of variation with regard to their shape and nature (Fig. 2). Sporulating sectors, mycelial sectors as well as mycelial patches were also observed. The colour of the mycelium and the substrate also varied considerably in different treatments. Though the shape of the sporulating sectors varied markedly, they had almost similar type of sporulation which was in the form of thin and slimy spore masses. In general, the higher treatment of MBA tended to form more of degenerated dark mycelial type with yellow pigmentation. Subcultures from different portions of the sectors on oat meal agar resulted in a number of variants. The most striking variant was obtained from the spores treated for 24 hours which showed profuse yellow pigmentation in culture and was selected for further studies. Single spore culture of this was designated as C_3. Another variant obtained from the series treated for 128 minutes showed abundant mycelial growth with no apparent pink spore masses. This was also purified and designated as C_4 for further comparative study.

* Cultures showing profuse dark cottony mycelial growth with sparse sporulation (Chona and Padwick, 1942).

The results of the comparative rate of germination and appressorial formation of the variants and the parent culture on yeast extract, (given below) showed that the percentage germination was low in the variants as compared to that of the parent whereas the appressorial formation was generally more in the former than the latter. Among the variants, C_4, which was a mycelial type resembling more or less the dark strain of *C. falcatum* and was picked up from the highest treatment of MBA, i.e. 128 minutes with 0.1 mM concentration, showed low percentage germination and appressorial formation. There was no appreciable difference in the size of conidia of different variants.

TABLE Comparative rate of germination of conidia and appressorial formation of the parent and the variants of *C. falcatum*.

Variant	% germination	% appressorial formation
C_1	50.6	62.5
C_2	63.5	78.3
C_3	67.3	97.2
C_4	23.6	9.0
C	91.0	14.0

The results of the growth studies (fig. 3) of the variants in comparison with the parent on two different media showed that the variants were comparatively slow growing on both the media. Variant C_3, which was obtained as a result of treatment with 0.1 mM of MBA for 24 hours and which showed profuse yellow pigmentation in culture medium, was the slowest growing as it covered only 6.4 cm. colony diameter even after 12 days of incubation on dextrose-asparagin-phosphate medium against 10 cm. in case of the parent and other variants.

It is observed that sectoring can be induced in *C. falcatum* with the aid of nitrogen mustard and concentration of 0.01 mM to 0.1 mM appears to be quite effective for the purpose. The efficiency of this compound in inducing mutation has also been reported by some other workers and it has been claimed (Stahmann and Stauffer, 1947) that the frequency of mutation in *Penicillium* due to nitrogen mustard was in the same range as that obtainable by ultra-violet radiation.

SUMMARY

The germination of conidia of *C. falcatum* Went was adversely affected as a result of the treatment with nitrogen mustard.

Pronounced sectoring was observed in the cultures raised from the spores treated with mustard gas. Red rot organism, thus, appears to be sensitive to the mutagenic effects of the chemical.

ACKNOWLEDGMENTS: The authors wish to express their sincerest gratitude to Dr. R. S. Vasudeva, Head of the Division of Mycology and

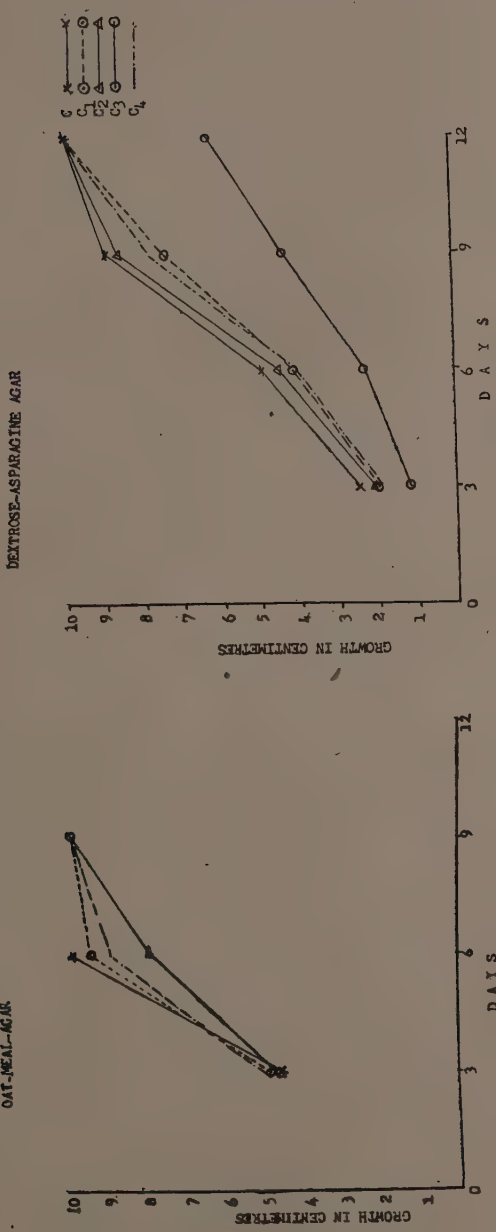
DEXTROSE-ASPARAGINE AGAR
OAT-MEAL-AGAR
GROWTH IN CENTIMETRES
DAYS
0
3
6
9
12
1
2
3
4
5
6
7
8
9
10
C
C1
C2
C3
C4

Fig. 3.

Plant Pathology for suggesting the problem, guiding throughout the investigation and going through the manuscript. Thanks are also due to Dr. B. L. Chona, Sugarcane Mycologist, for the keen interest he has evinced in the problem by giving helpful suggestions.

Division of Mycology and Plant Pathology,
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PURIFICATION OF BOTTLE GOURD MOSAIC VIRUS

G. P. S. Anand

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A mosaic disease of bottle gourd, (*Lagenaria siceraria* Standl) has been of common occurrence in Delhi for the last many years. The disease has been described by Vasudeva *et al.* (1950, 1954) and has been designated as *Cucumis* Virus 2C or *Marmor astrictum* var. *subobscurum* var. nov.

Different methods of isolation by chemical means with some modifications were tried and the procedure described herein was found to be successful.

Young leaves showing typical symptoms of the disease were collected from the diseased plants, frozen overnight, thawed and minced in a pestle and mortar. In order to get a regular supply of young infected leaves, the leaves were regularly harvested every third day as the preparations from old leaves usually contained lot of colouring matter which could not be removed during further purification process. The juice was expressed from the minced leaves by hand through a double layer of muslin. To the leaf residue a small amount of double distilled water was added, the residue minced and the juice expressed through muslin for the second time. The two lots were mixed together. Sufficient ethyl alcohol was then added to make it 30% alcoholic and the resultant mixture left at room temperature for two hours for precipitation of bulk of the plant constituents. The precipitate was removed by centrifuging at 2,000 r.p.m. for 20 minutes. The light green supernatant was treated with an equal volume of absolute ethyl alcohol and the mixture left at room temperature for two hours. This resulted in a light brown precipitate which contained the virus. This was centrifuged off at 3,000 r.p.m. for 20 minutes. The supernatant was discarded and the precipitate taken in a small amount of double distilled water. This was contrifuged again at 3,000 r.p.m. for 20 minutes and the supernatant collected. The residue was washed with small amount of double distilled water and this was repeated five times. All the washings were collected and mixed with the first supernatant. To the supernatant ammonium sulphate was added to make it one third saturated with the salt. The mixture was left at room temperature for two hours when a very fine whitish precipitate settled down at the bottom. The precipitate was collected by contrifuging at 3.000 r.p.m. for 20 minutes, suspended in a small amount of double distilled water and the pH adjusted to 7.0. The mixture was centrifuged at 2,000 r.p.m. for 20 minutes and the supernatant collected. The residue was washed with a small amount of double distilled water for five times and the supernatant from all these washings mixed with the first supernatant. The pH of the mixture was brought to 4.2 by adding N/10 HCl.

This resulted in the precipitation of the virus which was collected by centrifuging off the supernatant at 3,000 r.p.m. for 20 minutes. The virus precipitate was suspended in a small amount of double distilled water and its pH adjusted to 7.0 with N/10 NaOH. The precipitate dissolved in water at this pH. The solution was centrifuged at 2,000 r.p.m. for 20 minutes to remove the insoluble material. The virus was again precipitated from the supernatant at pH 4.2 with N/10 HCl. The virus precipitate obtained as above was taken in a small amount of double distilled water, pH raised to 7.0 with N/10 NaOH and the insoluble material removed by centrifuging at 2,000 r.p.m. for 20 minutes. The supernatant thus obtained was a pale, viscous solution containing the virus in a highly concentrated form. This was dialyzed in a cellophane paper bag against running tap water for 24 hours. Infectivity tests were conducted on healthy bottle gourd seedlings at each and every step of the treatment during purification and with the final purified preparation and positive results obtained.

The problem of formation of colouring matter during the purification process, presumably due to oxidation, was overcome later by an improvement in the above mentioned technique in the early stages of purification. This consisted of dipping the leaves in a bath of 2% sodium sulphite (A.R.) solution in double distilled water for half an hour, before freezing and thawing process. This enabled even the old leaves to be utilized for purification of the virus in bulk. The improved techniques yielded, more highly purified opalescent virus preparation than obtained by the former technique. Controls were set up wherein healthy leaves were harvested and treated in an identical way as described above. No virus precipitate was obtained at the stage when ammonium sulphate was added.

For electron microscopy a drop of virus solution was dried on a thin Formvar film mounted on silver coated copper mesh grid. This was then shadowed with palladium at an approximate angle of 15°. The electron micrograph of the material was taken through the kind courtsey of Director, National Physical Laboratory on a RCA, EMU-2A model electron microscope. As shown in the figure the virus consists of rod shaped particles with an approximate width of 25 mμ and a variable length of 100 mμ to 1350 mμ. This variation in the particle length might appear to be due to breakage or aggregation in linear succession of the virus particles during processing. However, a good number of particles have an average constant length of 800 mμ presumably to be the true length of the virus particles.

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology under whose guidance this work was carried out. Thanks are also due to Dr. C Dakshinamurti and Mr. S. C. Mehta of the Chemistry Division for their help in preparing the copper mesh grids for mounting the virus material and to Dr. Kanwar Bahadur of the National Physical Laboratory New Delhi for taking the electron micrographs of the purified virus preparation.

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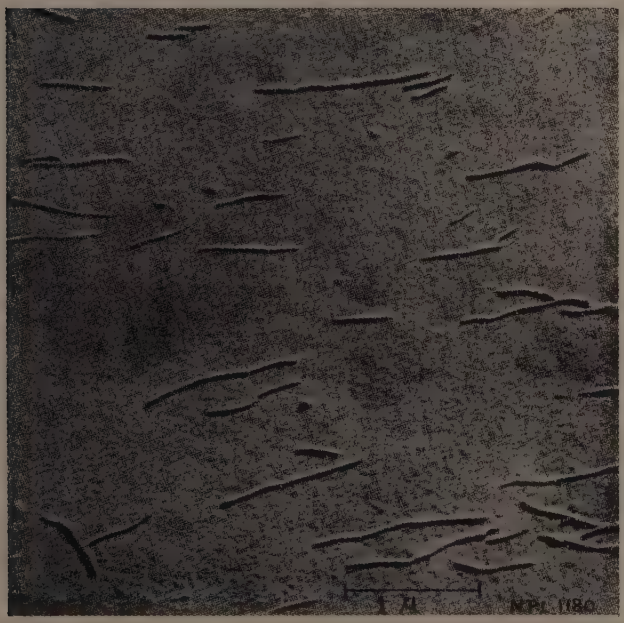

Electron micrograph of purified Bottle gourd mosaic virus.

VARIATIONS IN COLLETOTRICHUM FALCATUM WENT, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE

B. L. Chona and D. N. Srivastava

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Introduction. Red Rot disease caused by *Colletotrichum falcatum* Went is the most serious disease of sugarcane. Though the disease is known to have occurred in India from time to time since 1902, it attracted special attention during the years 1938-41 when it appeared in a very severe epidemic form in North Bihar and Eastern Uttar Pradesh, the main sugarcane tract of the country, affecting chiefly the then pioneer commercial cane variety, Co. 213, which had served effectively the Indian sugar industry for a period of about a decade. The cane-crush of most of the factories in the affected areas was reduced to one-third of the normal during 1938-39 and about one-half during 1939-40.

The disease was reported again in a severe form during 1946-47 from several districts of U.P., mostly affecting the popular variety, Co. 312, which met the same fate as its predecessor, Co. 213. In 1948-49, red rot was fairly widespread in Eastern U.P. as well as Mid-Eastern and certain parts of Western U.P. and amongst the affected varieties was Co. 453, a variety released for general cultivation after careful testing for resistance to red rot. A severe localised outbreak of red rot was reported from Bihar during 1949-50, affecting Co. 313 which was claimed to be field resistant to the disease by virtue of its having escaped the 1938-41 epidemic, and which occupied the same status in Bihar as Co. 312 in U.P. In 1950-51, localised red rot epidemic affecting Co. 312, 313 and 421 was reported from Eastern Punjab. Isolations from diseased canes collected from these localities have throughout revealed the predominance of the light highly sporulating virulent strain of *C. falcatum*.

While extensive studies have been carried out on pathological aspects of the red rot disease, comparatively little work has been reported regarding the variations met with in the causal organism of the disease.

The first indication of the possibility of physiologic specialisation in *Colletotrichum falcatum* was given by Edgerton and Moreland (1920) who suggested that the differences in the strains of the organism might be one of the factors responsible for the divergent results regarding reported modes of infection in India and United States.

Abbott (1938 and 1946), in seeking the cause of the red rot epidemic in Louisiana in 1930-31 which resulted in the sudden failure of the most promising and chief commercial variety, P.O.J. 213, discovered the existence of two distinct morphological races of the fungus, a light coloured highly virulent strain and a dark coloured less virulent one. Isolates intermediate in character between the two races were also obtained. He

explained that the failure of P.O.J. 213 occurred due to a change from the previously existing less virulent dark strain to the new and more virulent type which dominated the epidemic. This was the first evidence showing that physiologic specialization did exist in *C. falcatum*. Besides, he made detailed cultural studies of numerous isolates from America and other countries.

Chona (1940-41) observed an exact parallel in the failure of Co. 213 (a progeny of P.O.J. 213) in Northern India. He ascribed the red rot epidemic and the failure of the then leading commercial variety, Co. 213, to the same cause as that described by Abbott in America for the failure of P.O.J. 213. He discovered the predominance of a light coloured race in epidemic tracts till then unrecorded in India.

The differences encountered in the cultural characters and virulence of *C. falcatum* isolates necessitated a systematic study of the variation existing in the fungus and the results are reported in this paper.

MATERIALS AND METHODS. Thirty two *C. falcatum* isolates were selected from the stock culture collection at the Indian Agricultural Research Institute. The selection comprised of almost all the morphologically distinguishable strains isolated from a number of important cane varieties growing in different parts of Northern India from time to time since the red rot epidemic of 1938-39. Oat meal agar medium was used for growing the cultures throughout the course of the present investigations.

To ensure purity of the isolates, monospore cultures of 21 isolates from which spore suspensions could be made conveniently were established. In case of the remaining 11 isolates, which showed few spores and from which conidial suspensions could not be made easily, single hyphal-tip cultures were made.

Monoconidial or hyphal-tip cultures of the 32 *C. falcatum* isolates grown on Oat meal agar for 20 days at 30°C were inoculated in Petri dishes 11 cm in diam. and each containing 40 c.c. of Oat meal agar. The cultures were allowed to grow at 30°C for 25 days and observations on cultural and morphological characters recorded during this time.

EXPERIMENTAL RESULTS

GROWTH RATE: Measurements of radial growth of the isolates made at intervals of 2, 4, 6, 8, 10 and 12 days showed that there was considerable variation in the rate of growth of different isolates. Ten isolates recorded radial growth of 10.5 cm. in 8 days, whereas 12 isolates attained the same amount of growth in 10 days and the remaining 10 isolates took 12 days. Maximum radial growth obtained in 6 days was 8.0 cm. in one isolate as against the minmum of 4.0 cm. in another.

CULTURAL CHARACTERS: In the study of cultural characters of the isolates such aspects as colour and texture of the mycelium, nature and degree of sporulation, shape of conidia, their curvature, mutication and

granulation were considered. On the basis of the colour of the mycelium, the isolates were broadly classified into "light" and "dark" types. Each of these were subdivided into the following groups and subgroups on the basis of extent and texture of the mycelium and the nature and degree of sporulation:

A. *Light type:* Mycelium whitish to light grey.

1. Highly sporulating: Mycelium sparse, loose, fluffy, forming abundant pink spore masses.

   a. Spores formed in prominent round pinkish masses with abundant acervuli and setae - 5 isolates.

   b. Spores formed in slimy pinkish masses without acervuli and setae - 2 isolates.

2. Sparsely sporulating: Mycelium usually abudant, felty, spores formed underneath the mycelial mat.

   a. Spores formed in a slimy pale pink layer - 4 isolates

   b. Spores formed in scattered incospicuous masses - 2 isolates.

3. Non sporulating: Mycelium cottony, abundant, spores very rare - 5 isolates.

B. *Dark type:* Mycelium dark grey to olivaceous black.

1. Highly sporulating: Mycelium sparse, cottony, loose, forming abundant pink spore masses in concentric rings - 1 isolate.

2. Sparsely sporulating: Mycelium dense, felty with spores formed in tiny inconspicuous dots - 10 isolates.

3. Non - sporulating: Mycelium dense and felty, spores very rare - 3 isolates.

It may be mentioned that although several groups could be distinguished within the species, there were always isolates intermediate in characters between the different types, groups and sub-groups.

A comparison of the cultural characters of the isolates as recorded at the time of their isolation from the host with those observed in the present studies, after maintenance on artificial culture media for several generations, showed that only 9 isolates remained stable in their characters and the remaining 23 isolates had undergone morphological changes giving rise to various forms. The change mainly consisted in the loss of sporulation to different degrees but the reverse has never been observed. It was found that frequent and monospore subculturing prevented this loss of

sporulation for an indefinite length of time in most of the isolates. This observation is in accord with that of Lucas (1943) who found that in old cultures patch variants are formed which on subculturing produced only few spores thus causiug deterioration of the cultures.

Experience has shown that isolations made from diseased canes from localities affected with the red rot epidemic invariably yield light, highly sporulating strains whether isolated from the diseased stalk or midrib lesions. Majority of these isolates form conspicuous pink spore masses of the size of a mustard seed comprising of acervuli and spores while some form a slimy pinkish thick layer of spores. The dark sparsely sporulating isolates are only rarely encountered in epidemic areas. On the other hand, when isolations are made from midrib lesions from canes in localities free from stalk infection, only the dark, sparsely sporulating isolates are encountered. The light, highly sporulating strains tend to deteriorate rapidly on culture media if frequent monospore subculturing is not resorted to.

SIZE OF CONIDIA: Measurements of conidia of 21 isolates, which sporulated freely, showed marked differences in their length in some of the isolates, the extremes being 10 and 36 μ. The average conidial length of the 7 isolates belonging to the light highly sporulating group was much greater than that of the 14 isolates belonging to the other groups, being 30 and 26 μ, respectively. While the maximum of 36 μ, obtained in these studies is within the limit of maximum conidial length of 48 μ recorded by Abbott (1938), the minimum of 10 μ obtained in one of the isolates is, however, hitherto unrecorded.

GERMINATION OF CONIDIA: Germination of conidia of 20 sporulating isolates was studied in distilled water. The germination counts of the conidia were taken after 24 hours' incubation at 30°C.

A very marked degree of difference in the germination percentage of the various isolates was observed, the minimum being 0 per cent in an isolate of the highly sporulating group as against the maximum of 80 per cent in another isolate belonging to the light sparsely sporulating group. On the whole, the germination of conidia of the 7 isolates belonging to the light, highly sporulating group was much lower than that of the remaining 13 isolates belonging to the other groups, the average being 13 and 53 per cent, respectively. Variations in temperature, use of the tap and rain water and longer incubation (48 to 96 hours) tried, failed to induce any increase in the germination percentage of any of the isolates. It is interesting to note that the conidia of the light highly sporulating isolates, where the germination was usually low, showed high degree of granulation. On the contrary, in case of the isolates of the other groups where the germination was high, the conidia were either transparent or only slightly granulate. The indication of such great deal of variation in the germination capacity of conidia of the various isolates, being of interest, led to further investigation in this respect and 90 to 100 per cent germination of conidia of these isolates was obtained in sterilised cane juice diluted with 3 parts of water after 24 hours' incubation at 30°C.

The conidia may germinate from one or both ends or from the middle region. The conidia after germination develop 1–2 septa, become empty and present a transparent appearance. Conidia of majority of the isolates of the light highly sporulating group produced one or more terminal appressoria on germination. Appressoria were, however, formed in a few isolates belonging to the other groups also.

Resistance to heat: Lethal temperature of 24 days old cultures of each of the 32 isolates, growing on oat meal agar at 30 °C, was determined. Though some of the isolates varied slightly in their resistance to heat, a great majority of them were killed between 51 and 54°C after 5 minutes exposure.

Virulence test: Inoculation experiment was carried out to compare the virulence of the 32 isolates on a highly susceptible cane variety, Co. 445, and also to determine it there was any correlation between any of the cultural, morphological, or physiological characters of the isolates and their virulence.

Using the standard plug method, pure cultures of each of the 32 isolates were inoculated separately into about 25 healthy standing canes of Co.445 planted in 32 separate plots. Uninoculated controls were also maintained. Observations were recorded after 6 months of making the inoculations, when the inoculated canes were split open longitudinally and the linear spread of infection, above and below the point of inoculation, recorded in inches.

Judging from the average linear spread of infection, wide variation in the virulence of the various isolates was observed. Maximum average length of infection of 73 inches was recorded in an isolate of the light highly sporulating group as against the minimum of 14 inches in an isolate of the dark sparsely sporulating group, while the controls did not show any infection. On the whole, the 7 isolates of the light highly sporulating group were found to be much more virulent than the 25 isolates of the other groups, the average infection being 41 and 21 inches, respectively.

The data relating to some of the experiments are summarised in Table I.

The data presented indicate that the germination capacity of conidia of the isolates is inversely related to their virulence. Further, in the case of the 7 isolates, beloging to the light highly sporulating group which recorded nearly twice as much of average infection as the isolates of the remaining groups, the conidia were granulate, but in the isolates of the other groups they were hyaline. More intensive work in this respect however, appears to be necessary.

TABLE I. Relationship of granulation and germination of Conidia of different groups of isolates of *C. falcatum* to their virulence

Group of Isolates	Granulation of Conidia	Germination of Conidia in water		Virulence on the host	
		*No. of isolates tested	Average germination (per cent.)	No. of isolates tested	Average linear spread of infection (inches)
I. Light highly sporulating	Granulate	7	13	7	41
II. Light sparsely sporulating	Transparent	5	67	6	22
III. Light non-sporulating	–	–	–	5	19
IV. Dark highly sporulating	Transparent	1	52	1	18
V. Dark sparsely sporulating	Transparent	7	42	10	23
VI. Dark non-sporulating	–	–	–	3	22

*Number of isolates studied for germination of Coinidia was 20 only.

DISCUSSION. The rate of growth of the isolates in culture, though it varies considerably, was not found to be correlated with their morphological characters. physiological behaviour or pathogenicity. This fact is in agreement with the observations of Abbott (1938).

The colour and texture of the mycelium and the nature and degree of sporulation appear to be interrelated to some extent with certain conidial characters as also with the virulence. For example, in case of the 7 isolates of the light highly sporulating group which showed high degree of virulence, the conidia were highly granulate but showed poor germination in water. Furthermore, the conidia of these 7 isolates were generally longer with smaller ranges of frequency than those of the isolates belonging to the other groups. Other conidial characters such as curvature, mutication and vacuolation vary within the same isolate to a considerable extent and were not found to be related to other characters. It is interesting to note that acervuli were formed in culture in 5 out of 7 isolates of the light highly sporulating group. Their absence from the isolates of other groups shows that this character is also related to some of the morpological characters as also the virulence.

Even within the light highly sporulating group, there was considerable variation in the virulence of the different isolates. It is, therefore, necessary to exercise great care in the selection of *C. falcatum* isolates for varietal resistance tests. The most virulent isolates must be picked up in a preliminary screening test on the host and maintained in its virulent form by frequent subculturing and single-sporing.

Summary

The cultural, morphological and physiological studies, and the virulence tests carried out reveal the existence of variously differentiated forms of *Colletotrichum falcatum* Went in India.

A comparison of the original characters of the isolates at the time of isolation and their later morphological characters showed that a majority of *C. falcatum* isolates are unstable and subject to variations on culture medium.

Comparative growth-rate studies of the isolates under identical conditions on Oat meal agar showed considerable variation in many of the isolates, but no correlation of the rate of growth was observed with the morphological characters, physiological behaviour or virulence.

On the basis of the cultural characters, the isolates have been divided into 6 groups. Colour of the mycelium, degree and nature of sporulation, granulation of conidia, their germination capacity and virulence on the host appear to be closely related.

Conidial length varied considerably in the 32 isolates of *C. falcatum* studied, the lowest extreme being 10μ and the maximum 36μ.

Germination of conidia showed a very marked degree of variation between the isolates of different morphological groups and it appears to be inversely related to the degree of granulation and the virulence on the host.

The virulence of various isolates on the cane stalk showed a very wide range of variation. The light highly sporulating isolates were found to be more virulent than isolates of the other groups.

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Reperences

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PHYSIOLOGIC SPECIALIZATION IN PUCCINIA HORDEI

R. S. Vasudeva, L. M. Joshi and K. R. Sreekantiah

(Accepted for publication December 20, 1959)

Leaf rust or dwarf rust of barley caused by *Puccinia hordei* Otth. [*P. anomala* Rostr. = *P. simplex* (Koern.) Erikss. and Henn.] was first reported from India in 1918 by Butler. He recorded it as a rare rust in this country. The rust has apparently not been recorded since then though it was collected from time to time from West Bengal and Bihar and was presumed to have been restricted to that area alone. In March 1955, it appeared in Delhi in somewhat virulent form attacking a large number of varieties in experimental plots. On the whole the infection was mild and the intensity of attack varied from traces to light. The rust was again observed in the following year on some varieties of barley but was just in traces. In February-March 1958, the incidence of the rust was heaviest so far observed at Indian Agricultural Research Institute fields. Out of three hundred and ninety-eight varieties, one hundred and sixty were rust infected. Amongst these varieties B.21, B.37, B.41, B.42, B.47, B.48, B.54, B.61, B.71, B.80, B.148, B.149, B.151, B.178, B.180, B.182, B.207, B.219, B.221, B.228, B.240, B.250, B.259, B.260, Afg. x C.59 and Afg.12, showed light to moderate infection. Barley varieties, B.222, B.223, NP 13 and NP 21 in particular deserve special mention as they showed very heavy infection of the rust. The remaining varieties showed just traces of the rust that too only on the lower leaves of the plant.

A sudden sporadic outbreak of the rust has naturally led to an extensive search in other parts of the country. In 1955, the year when rust was first reported at I.A.R.I., it was collected from Raya and Kanpur (Uttar Pradesh) and Ketty (Nilgiri hills in Madras State). In subsequent years the rust has been collected from Delhi, Uttar Pradesh, Bihar and Madras States. It is, therefore, obvious that the rust has a wider distribution than was considered hitherto. So far the disease has not assumed a serious epidemic form and the losses caused by it have not yet been assessed.

As the rust is gaining prominence and is more widely spread than considered hitherto, some preliminary work on the life cycle of the fungus and physiologic specialization has already been initiated (Joshi *et al* 1959). So far attempts to germinate the teleutospores have not been successful but the work is in progress. In the mean time work on the identification of races has been taken up. For this work sixteen differential barley varieties selected by Levine and Cherewick (1952) have been used. Joshi *et al* (1959) analysed a few samples and based on the reactions of the differential varieties recorded a new race. Further studies of these samples, using some exotic and local varieties revealed the presence of at least a biotype of the same race. As the race did not resemble any of the races

listed in the key of Levine and Cherewick (1952) it was provisionally designated as H1 and its biotype as H1-A.

Since then work on study of physiologic races is being continued and during 1957-58 a few samples collected from different parts of the country were analysed. All the samples except one from Anikorai (Nilgiri Hills) revealed the presence of the same race as was recorded in previous years. The Anikorai sample yielded two races out of which one was new and different from the previously recorded race by its reactions on variety Austral. The reactions of all the differentials to this isolate are given below in a tabular form.

TABLE: Reactions of differential hosts to new race of *P. hordei*.

DIFFERENTIAL HOSTS																
Speciale	Reka	Sudan	Bolivia	Oderbrauker	Quinn	Egypt-4	Gold	Letchtaler	Cruzat	Chilean-D	Club Mariout	Samaria	Berg	Austral	Kinver	Local
4	0–1	4	0–1	4	0	3–4	0;	0;	3–4	4	4	4	3–4	3–4	4	4

Since the races do not resemble any of the races listed by Levine and Cherewick (l.c.), they both have been designated as *Puccinia hordei* race H1 and *P. hordei* race H2 (H1 and H2, respectively). The biotype of the former has been designated as H1-A. It may, however, be mentioned here that race H1 shows close resemblance to race 2 in its ractions on all the differentials excepts for the reactions on varieties Quinn and Letchtaler where some minor differences are observed. It is not unlikey that under identical conditions the difference might not be so significant as to call it distinct from race 2.

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STUDIES IN THE MUCORALES
II. A NEW SPECIES OF MORTIERELLA FROM INDIA

B. S. Mehrotra

(Accepted for publication December 15, 1959)

The genus *Mortierella*, though very common in several parts of the world, is practically unknown in India except for a single report by Subramanian (1952) who has listed *Mortierella ramanniana* var. *angulispora* (Naumov) Linnemann in his list of soil fungi of Madras. During the course of isolations of soil fungi from a heavily manured garden soil of Allahabad the author came across a species of *Mortierella* which is apparently not identical with any member of this genus hitherto described, and for which the following name is proposed based on the country of origin:

Mortierella indica sp. nov.

Colony on hay-extract agar and soil-extract agar consisting of thin, wide-spread, colourless, much branched and irregularly swollen submerged mycelium and ramifying aerial mycelium (branching at an angle of approx. 60°). On potato-dextrose agar the colony consists of much aerial mycelium, showing a lobed appearance. Sporangiophores seen only on hay-extract and soil extract agar, 100–385 μ, attached at the base by slightly brown to colourless rhizoids, swollen right at the base (not narrow at first), gradually tapering from 8–13 μ at the base to 2.8–3.2 μ at the apex with a bulged transverse septum at the tip, 3–3.5 μ in height; often branched, ramification cymose, branches 42–65 μ long, 2.8 μ at the base to 1.4 μ at the apex. Sporangium 14–22 μ in diameter, colourless, leaving a collar on the sporangiophore on dehiscing, about 50 or slightly more sporangiospores in each sporangium. Sporangiospores elliptical to oval, very rarely globose; smooth, 4–11 x 3–4 μ (average 6 x 3 μ) in size.

Stylospores intercalary and terminal, 20–30 μ in diameter, borne on a single or on double stalks, echinulate to spiny, spines long 3–6 μ (generally 5 μ).

Zygospores not seen.

Type locality: Botanical Gardens, Allahabad University, India.

In agaro extracti graminacei et in agaro extracti humi coloniae constante mycelio tenui, late diffuso, incoloro, abundanter ramoso, irregulariter tumescente, submerso et e mycelio aereo ramoso (ramis productis ad 60° plus minusve). In agaro dextrosi Solani tuberosi coloniae constante mycelio multo plus aereo, et lobatae apparent. Sporangiophori noti tantum in agaro extracti graminacei et agaro extracti humi, 100–385 μ, ad basin fixi rhizoideis tenuiter brunneis vel incoloris, tumescentes ad ipsam basin (primo non angusti), gradatim fastigati ex 8–13 μ

1 Subramanian, C. V. (1952) J. Madras Uni. **22 B**: 206-222

in basi, ad 2.8–3.2 μ in ipso apice, ornati septo tumescente transverso ad apicem—3–3.5 μ, alti, saepe ramosi, ramis cymosis, 42–65 μ longis, 2.8 μ ad basin 1.4 μ ad apicem. Sporangium 14–22 μ diam., incolorum, post dehiscentiam annulo relicto in sporangiophoro, ca. 50 vel paulo plures sporangiospori in singulis sporangiis. Sporangiospori elliptici vel ovalis, rarissime globosi leves, 4–11 x 3–4 μ (mediet. 6 x 3 μ magnitudine). Stylosporae intercalares et terminales, ca. 20–30 μ diam., insidentes singulis vel binis pedicellis, echinulatae vel spinosae, spinis longis, 3–6 μ (ut plurimum 5 μ). Zygosporae non visae. Locus typicus: hortus botanicus universitatis Allahabadensis, in India.

A culture of this species has been deposited at ARS Culture Collection, Fermentation Laboratory, Northern Utilization Research and Development Division, Peoria, Illinois, U.S.A., and Culture Collection, Botany Department, University of Allahabad.

Like a number of other species of *Mortierella*, this species also shows the characteristic lobed appearance on potato-dextrose agar medium (Plate I, fig. 1). It can be placed in the Section Polycephala of the key to the species of *Mortierella* which includes species having both stylospores and sporangiospores and in which the sporangiophores are usually borne on the aerial mycelium. The only species to which the present one comes close is *M. polycephala* or its two known varieties but with them also there are substantial differences to justify giving a new specific name for this isolate. The sporangiophores here are cymosely branched (Text fig. 2 and 3) but are racemosely branched in *M. polycephala;* they range from 100–385 μ in size, are swollen right at the base and have well defined rhizoids (Text-fig. 4–6), but in *M. polycephala* they are 250 μ in length, generally swell to their full size above their base and the rhizoids are either partly formed or are altogether absent. Besides this, the sporangiophores in the present species have a bulged transverse septum at the apex (Text-fig. 7; Plate I, fig. 2). In *M. polycephala* the septum at the apex of the sporangiophore has been figured as plane. The sporangia are much smaller in size ranging from 14–22 μ as against 30–70 μ in *M. polycephala*. The sporangiospores, in this species are elliptical to oval in shape (Text-fig. 8; Plate I, fig. 2) but in *M. polycephala* they have been described and figured as oval and spherical. Further the average size of the sporangiospores here is smaller being 6 x 3 μ while in the case of *M. polycephala* they are 10–12 μ in size. Lastly the stylospores here are echinulate to spiny, spines ranging from 3–6 μ (average 5 μ), intercalary as well as terminal (Text-fig. 9–11; Plate I, fig. 2). and are sometimes borne on a double stalk (Text-fig. 11). Such stylospores have never been seen in any other species of *Mortierella*.

Acknowledgments. The author is much indebted to Dr. G. Linnemann and Dr. C. W. Hesseltine for their encouragement and valuable suggestions; and to Rev. Fr. Dr. H. Santapau for rendering the Latin diagnosis. Thanks are also due to the Scientific Research Committee, U.P. for the contingent grant.

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PLATE I.

Fig. 1. Colony of the fungus on potato-dextrose agar. 7 days.

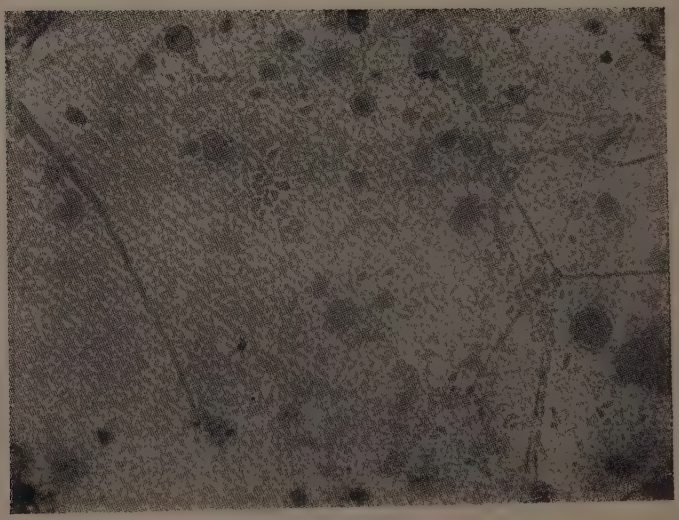

Fig. 2. Photomicrograph of a slide mount of the fungus showing the sporangiophores (Right and Left), sporangioshores (Centre), and stylospores (scattered) Approx. x 300).

EXPLANATION OF TEXT-FIGS. 1–11

Fig. 1. Semidiagrammatic sketch to show the relative position of the sprangiophore and mycelium.

Fig. 2. Top portion of a sporangiophore with two branches (x 1000).

Fig. 3. A complete sporangiophore with three branches (x 240).

Figs. 4-6. Bases of three sporangiophores showing rhizoids (x 1000).

Fig. 7. Apex of a sporangiophore showing the bulged septum (x 2000).

Fig. 8. Few sporangiospores (x 1600).

Fig. 9. A terminal stylospore (x 1600).

Fig. 10. An intercalary stylospore (x 1600).

Fig. 11. A stylospore on a double stalk (x 600).

STUDIES ON POWDERY MILDEWS FROM INDIA-1.

B. L. Chona, J. N. Kapoor and H. S. Gill

(Accepted for publication December 30, 1959)

Recently, studies on Indian Erysiphales have been taken up in the Division of Mycology of the Indian Agricultural Research Institute, New Delhi. Critical examination of collections made from different parts in the Himalayas as also those made locally have revealed some interesting features. The present paper gives an account of one new species, two new records and seven new host records for India. These specimens have been deposited in the Herb. Crypt. Ind. Orient., Indian Agricultural Research Institute, New Delhi and their accession numbers are indicated in the text.

1. *Erysiphe sikkimensis* spec. nov.

Epiphylla, efformans maculas albidas irregulares, saepe totam follii paginam operienns, tandem evadens sordide alba. Mycelium hyalinum, septatum, 4 - 8 μ latum; conidia granulata, ellipsoidae vel doliiformia, hyalina, 25 – 40 x 14 - 18 μ; perithecia dispersa, brunnea vel fusce brunnea, sub-lenticularia, globosa vel sub-globosa, parva, 54 – 79 μ diam.; cellulae 7 – 14 μ latae; appendices sparsae, hyalinae vel subhyaliae, septatae, flexuosae, breves, plus minusve aequantes diametrum peritheciorum vel hunc superantes, aliquantum irregulariter ramosae; asci 4–5, sub-globosi, vel late ovati, breviter pedicellati, 40 – 50 x 32 – 43 μ; ascosporae 6-7, hyalinae, oblongae, unicellulatae, 16 - 18 x 9 - 11 μ.

Typus lectus in foliis viventibus *Castanopsidis triguloidis* A. DC. (H.C.I.O. 26083 Typus) et *C. indicae* A. DC. (H.C.I.O. 26084 familia Fagacearum, ad partes orientales Sikkim, die 12 aprilis anni 1957 a J. N. Kapoor.

Erysiphe sikkimense spec. nov.

Epiphyllous, forms white irregular patches, often whole leaf surface is involved, later on becoming dirty white; mycelium hyaline, septate, 4 - 8 μ wide; conidia granulate, ellipsoidal to barrel-shaped, hyaline, 25 - 40 x 14 - 18 μ; perithecia scattered, brown to dark brown, sublenticular, globose, small, 54— 79 μ in diameter; cells 7 - 14 μ wide; appendages hypha like, sparse, hyaline to sub-hyaline, septate, flexuous short, about equalling or slightly more than the diameter of the perithecia, somewhat irregularly bent, sometimes branching; asci 4-5, sub-globose to broadly ovate, shortly stalked, 40—50 x 32 - 43 μ ; ascospores 6-7, hyaline, oblong, one-celled, 16-18 x 9-11 μ.

On living leaves of *Castanopsis tribuloides* A. DC. (H. C.I.O. 26083 Type) and *C. indica* A. DC. (H.C.I.O. 26084) Fagaceae, Eastern Sikkim, 12–4–1957 (J. N. Kapoor).

2. *Erysiphe umbelliferarum* de Bary in Beitr. Z. Morph. d. Pilze **1** : 50, 1870; Blumer, Zur Kryptogamenflora der Schw. Band 7, Heft 1, p. 195, 1933.

Conidia solitary, cylindrical, 30-42 x 12-18 μ; perithecia globose to sub-globose, 94-119 μ in diameter; cells indistinct; appendages numerous, irregularly branched and bent, septate, brown coloured; asci 3-8, 52-63 x 28-42 μ, ellipsoidal to globose, 3–6 spored; ascospores 17–21 x 10-12 μ.

On living leaves, leaf-sheaths, stems and fruits of *Daucus carota* L. (Umbelliferae) H.C.I.O. 25922, New Delhi, 10–5-1958 (H. S. Gill and Amar Singh)

The mildew attack was very severe on the carrot crop reserved for seed in the Botanical area of I. A. R. I., New Delhi.

3. *Microsphaera alni* (Wallr.) Winter; Rabenh. Krypt. Fl. Deutsch. **1** (2) : 38, 1884.

Conidia solitary, granulate, ellipsoidal, 25-39 x 18-32 μ in diameter; cells 11-21 μ wide; appendages variable in number, 1-3 times the diameter of the perithecia, amber brown at the base, 1-2 septate, apex 2-4 times dichotomously branched, tips of ultimate branchlets regularly and distinctly recurved; asci 47-57 x 32-47 μ , globose to ovate-globose, 4-8 spored; ascospores hyaline, 18-21 x 11 μ, ellipsoidal or oblong.

On living leaves of *Juglans regia* L. (Juglandaceae) H. C.I.O. 25903, Kulu, Punjab, 29—11—1957 (H. S. Gill)

4. *Phyllactinia corylea* (Pers.) Karst. in Act. Soc. Faum. Fl. Fenn. **2** : 92., 1885; Salmon, Monograph Erysiphe p. 224, 1900.

Conidia solitary, clavate to broadly clavate, vacuolate, granulate, 50-60 x 18-21 μ ; perithecia large, globose to sub-globose, 180-288 μ in diameter; cells rather obscure; appendages 5-16 in number, 1-3 times the diameter of the perithecia, rigid, acicular, straight, aseptate, colourless, swollen at the base into a hollow bulb; asci many, sub-cylindrical to ovate oblong, 64-93 x 25-36 μ more or less stalked, 2-spored, rarely 3-spored; ascospores 25-39 x 14-21 μ, oblong or ovate.

On living leaves of *Ulmus* sp. (Ulmaceae) H.C.I.O. 26082, Jogindar Nagar, H. P., 3-12-1957 (H. S. Gill); on *Salix* sp. (Salicaceae) H.C.I.O. 25905, Kulu, Punjab, 29-11-1957 (H. S. Gill)

5. *Sphaerotheca fuliginea* (Schlecht.) Pollacci in R. Ist, Bot. Univ. Pavia p.8, 1905; Sacc. Syll. Fung. **22** : 20, 1913.

Conidia produced in chains, elongate-ellipsoidal, 25-39 x 14-18 μ; perithecia globose to depressed-globose, 82-108 μ in diameter; cells very big and clear, 11-29 μ wide; appendages variable in number and length,

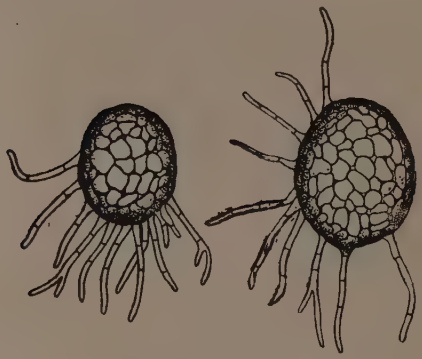

Fig. 1

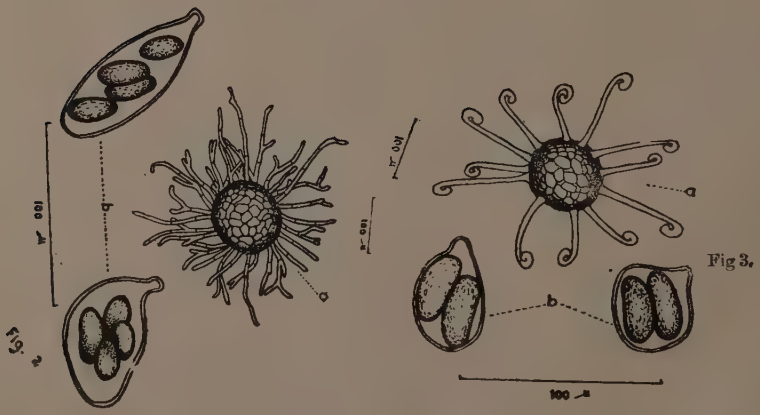

Fig. 2

Fig 3.

EXPLANATION OF PLATE

Fig. 1. *Erysiphe sikkimense*
a. perithecia
b. Asci and Ascospores

Fig. 2. *Erysiphe umbelliferarum*
a. Perithecium
b. Asci and Ascospores

Fig. 3. *Uncinula clandestina*
a. Perithecium
b. Asci and Ascospores

brown coloured throughout, simple, usually aseptate; ascus single, hyaline, ovate or sub-globose, with or without stalk, 65-83 x 54-68 μ, 5-8 spored; ascospores 18-21 x 11-14 μ, mostly 21 x 14 μ.

On living leaves and stems of *Cosmos* sp. (Compositae) H.C.I.O. 25900, Palampur, Punjab, 5-12-1957 (H. S. Gill and V. S. Sharma)

6. *Uncinula clandestina* (Biv. Bern.) Schroet. in Cohn's Krypt. Fl. Schles. **3** : 245, 1893; Salmon, Monograph Erysiphe p. 97, 1900.

Hypophyllous; perithecia in patches, also scattered, globose to sub-globose, 72-94 μ in diameter; cells 16-21 μ wide appendages 8-19, 1-2 times the diameter of the perithecia, apex helicoid, flexuous, aseptate, colourless; asci 3-5, ovate to sub-globose, with or without stalk, 39-47 x 29-36 μ, 2-spored; ascospores 21-29 x 14-18 μ, sometimes slightly curved.

On living leaves of *Ulmus* sp. (Ulmaceae) H.C.I.O. 25908, Kulu, Punjab, 29-11-1957 (H. S. Gill)

7. *Uncinula salicis* (DC.) Wint. in Rabenh. Krypt. Fl. Deutschl. **1** (2) : 40, 1884; Salmon, Monograph Erysiphe p. 81, 1900 and Bull. Torr. Bot. Club. **29** : 96, 1902.

Conidia solitary, ellipsoidal, 29-36 x 14-18 μ; 'perithecia globose to sub-globose, 122-162 μ in diameter; cells 7-18 μ wide; appendages 20-39 in number, 1-3 times the diameter of the perithecia, simple, aseptate, hyaline, apex uncinate; asci 8-12, elliptic oblong, broadly ovate, usually stalked, 64-86 x 36-50 μ, 6-8 spored; ascospores 18-29 x 10-14 μ, ellipsoidal.

On living leaves of *Populus alba* L. (Salicaceae) H.C.I.O. 25902, Kulu, Punjab, 1—12—1957 (H. S. Gill)

Our grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his keen interest, helpful criticism and providing necessary facilities for work. We also thankfully record the help of Rev. Father Dr. H. Santapau, Head of the Biology Department, St. Xavier's College, Bombay for rendering the latin diagnosis of new species.

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POLYPORACEAE OF THE MUSSOORIE HILLS - I

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Introduction. Under the leadership of Prof. P. N. Mehra an excurison is made every year from the Botany Department of the Punjab University to the Mussoorie Hills in the North-Western Himalayas (2,000–7,000 ft. altitude) to study the cryptogamic flora. The polyporaceae are part of the programme undertaken by Dr. K. S. Thind and his students. This first contribution describes seven known species and one new variety. The fruit bodies have been described from fresh material supplemented with dry material and that preserved in alcohol-formalin. The numbers of the species are the serial numbers of the polypores.

Bose, 1918–1942, has been an active student of Bengal Polyporaceae and has described in all 136 known species and 9 new species from Bengal. However, this important group of fungi has not received adequate attention from other regions of India. Bagchee and Bakshi (1950), Bakshi and Bagchee (1950), and Bagchee, Puri, and Bakshi (1954), have described diseases of Oaks and other hardwood trees in India caused by 38 species of Polyporaceae. In all, over 300 species of Polyporaceae have been recorded already from India, which is a very good representation of this group, although some of these may be synonyms since the reports of most of these are very old (Butler and Bisby, 1931; Mundkur, 1938). Between 1942 and 1956 only fifty-five species of Polyporaceae have been recorded from different parts of India as listed by Ramakrishnan and Subramanian (1952), and Subramanian and Ramakrishanan (1956). Thus, about 250 species have been reported before 1942.

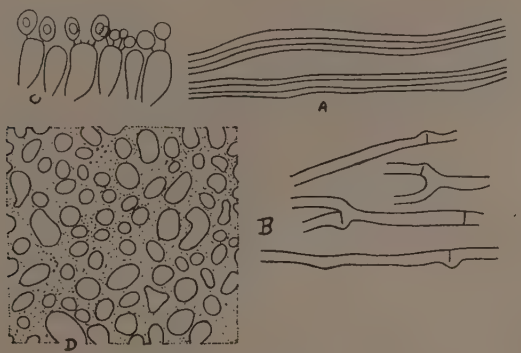
1. *Poria versipora* (Pers.) Baxter, Pap. Michigan Acad. Sci., Arts, Lett. **25** : 150. 1940.

(*Poria versipora* (Pers.) Rom., Sv. Bot. Tidsk., Bd. 20, H. 1, p. 15. 1926).

Syn: *Polyporus versiporus* Pers., Myc. Eur. **2** : 105. 1825.

Hymenophore annual, entirely resupinate, fleshy-tough when fresh, coriaceous and brittle when dry, easily separable from the substratum when fresh, usually not quite separable when dry, spreading over large areas on the substratum. *Hymenial surface* even, deep cream coloured, not fading on drying. Margin wavy, thin, lighter coloured, sterile 1–2 mm. wide. Dissepiments not toothed. *Pores* not in strata, rounded, daedaloid or irregular due to coalescence, deep cream coloured in section, up to 1.5 mm. deep, straight or obliquely placed, 90–158 μ in diameter, or 5–6 per mm. Dissepiments 26–72 μ thick, equal, of compact parallel hyphae, at apex finely and densely velutinate. *Context* deep cream coloured, 112–225 μ thick, of lax interwoven hyphae. *Hyphae* dimitic both in the context and trama. Skeletal hyphae long, brown in a mass.

subhyaline individually, aseptate, unbranched, 2.4–4 μ wide, wall 0.8–1.6 μ in thickness. Generative hyphae hyaline, thin walled, or only slightly thick walled, septate, branched, clamped, clamps abundant, 2–3.6 μ wide. Subhymenial hyphae composed of several rows of hyaline, short cells. *Basidia* clavate type, oblong to clavate, short, subhyaline, 7–12 x 2.8–4.0 μ. Sterigmata 4, nearly straight, 0.8–1.2 μ long. *Basidiospores* hyaline to subhyaline, very small, subglobose to ovoid or ellipsoid, smooth, uniguttate, guttule filling about one-third of the spore cavity, 2.4–3.6 x 2–2.4 μ. (Plate I, Fig. 2., Text-Fig. 1, A-D.)



Text—Fig. 1. *Poria versipora* (Pers.) Baxter,
A. Skeletal hyphae, x 750.
B. Generative hyphae with clamps, x 750.
C. Basidia with basidiospores x 1150. D. Pores, x 20.

Collected on logs of wood, Nala Pani, Dehra Dun, August 7, 1954, **196**. New record in India.

This Mussoorie collection closely resembles *Poria versipora* (Pers). Baxter in all microscopic structures including the very characteristic swollen hyphal endings in the tube walls which are capped by a large oily deposit. Pores are rounded but later on coalescing and becoming daedaloid or irpiciform, hence the name *versipora* for the species. The spores of the Mussoorie collection were not fully matured at the time of collection and hence rather very small for the species. Further collections will be made to clarify this point.

2. *Ganoderma applanatum* (Pers. ex Wallr.) Pat., Soc. Mycol. France Bul. **5** : 67. 1889.

(= *Fomes applanatus* (Pers. ex Wallr.) Gill., Champ. France **1** : 686. 1878.

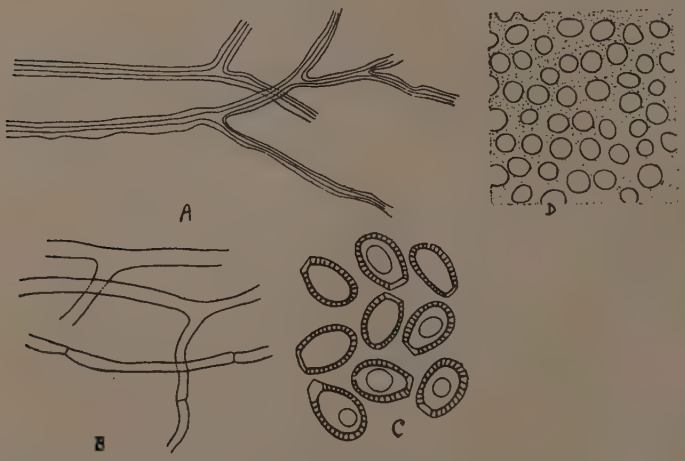
Syn. *Boletus applanatus* Pers., Obs. Myc. **2** : 2. 1799.

Polyporus applanatus Pers. ex Wallr., Flora Crypt. Germ. **4** : 591. 1833.

Polyporus megaloma Lev., Ann. Sci. Nat. Bot. III, **5** : 128. 1846.

Elfvingia megaloma (Lev.) Murr., Torrey Bot. Club Bul. **30** : 300. 1903.

Hymenophore annual, solitary, or imbricate, firm and woody. *Pileus* applanate, sessile or base of pileus modified into stalk like portion, 3.5–10 x 3.5–11 x 1.5–3.3 cm.; surface whitish brown, smooth, concentrically zonate, zones are more prominent towards periphery. Surface of pileus covered with hard and horny crust, crust cracked with age, hairs absent. Margin thick, obtuse, entire to wavy, concolorous. *Hymenial surface* plane, white when fresh, turning pallid on drying, even; margin concolorous, sterile, 0.5–1 cm. wide. Dissepiments not toothed. *Pores* not in strata, rounded, whitish brown in colour, 90–142 μ in diameter or 4-5 per mm. Dissepiments 52–187 μ thick, equal, edges not concolorous, white. *Context* chocolate brown, upto 1.5 cm. thick, of interwoven hyphae, concentrically zonate, consisting of alternate dark and lighter coloured bands of about equal width, fibrous. *Hyphae* dimitic, both in the context and trama. Skeletal hyphae brown to deep or dark brown, branched, branches attenuated at their apices into long, fine, paler portions, aseptate, 2.5–6 μ wide, thick walled, walls 0.8–2 μ thick, sometimes more thickened so as to obliterate the whole of lumen, minute hyaline bodies are present in the lumen of the hyphae, and they are widely spaced, do not take stain. Generative hyphae hyaline, branched, septate, 1.5–2.5 μ wide, thin walled, take stain. *Basidia* broadly clavate, subhyaline, soon collapsing, only a few basidia observed, 6–9 μ in diameter. *Basidiospores* ovoid with truncate apex, brown in colour, double walled, inner wall thick and echinulate, outer wall smooth, some spores have a guttule, 4.5–8 x 4–5 μ. (Text-Fig. 2, A-D.)



Text—Fig. 2. *Ganoderma applanatum* (Pers. ex Wallr.) Pat.
A. Sketetal hyphae, x 480. B. Generative hyphae, x 750.
C. Echinulate basidiospores truncate at one end, x 1150. D. Pores, x 20.

Collected on logs of wood, Saharanpur Road, Dehra Dun, August 5, 1953, **197.**

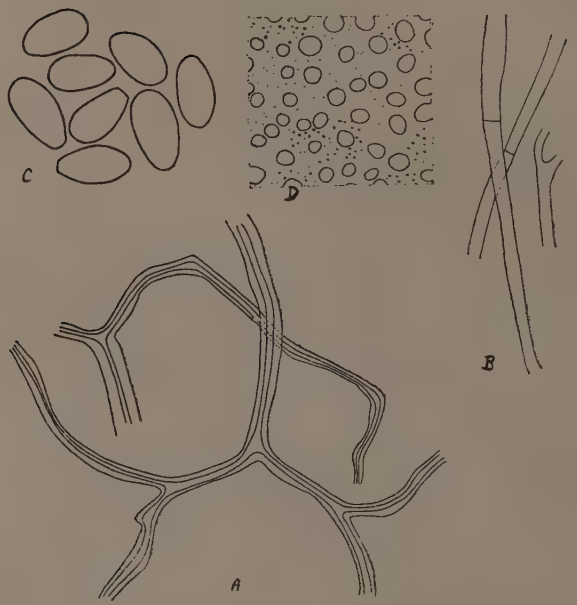
This Mussoorie collection undoubtedly belongs to *Ganoderma applanatum* (Pers. ex Wallr.) Pat. (=*Fomes applanatus* (Pers. ex Wallr.) Gill.) and is characterized by the appalanate fruit bodies covered over by a typically hard and horny crust not indentable with thumb nail, and ovoid, truncate brown, echinulate basidiospores, 5–8 x 4–5 μ.

Lowe (1953) in his Monograph (p. 100) has referred to two forms of this species—one with a soft but distinct brown crust and the other with a very hard and horny whitish or grey crust. The second of these has been known as *Ganoderma leucopharum* (Mont.) Pat. The Mussoorie collection (n. 197) possesses hard and horny, whitish crust and evidently is the form often known as *G. leucopharum* (Mont.) (=*Fomes leucopharus* Mont., Syll. Crypt., p. 157. 1856).

3. *Ganoderma applanatum* (Pers. ex Wallr.) Pat.

var. *laevisporum* Humphrey, Philippine Jour· Sci. **45** : 533. 1931.

Hymenophores perennial, new pilei arising below those of the previous year, solitary, dimidiate, hard and woody. *Pileus* applanate, 7–15 x 8.5–33 x 6–11 cm., sufrace dark brown to greyish black, uneven or rough, azonate, rimose with age, covered over by a hard, thick crust which is 1 mm. thick and blackish in section, hairs absent. Margin acute to subobtuse, entire, concolorous. *Hymenial surface* plane, dull white, turning brown or greyish brown on drying, previous year's hymenia turning black and get cracked, even, margin concolorous, on drying remaining lighter coloured at the edge, with a narrow sterile margin, 0.5–2 mm. wide. Dissepiments not toothed. *Pores* stratose, pore layers marked by a thin layer of context, rounded, brown in section, upto 2 cm. deep, tubes of different strata are of unequal depth, straight, old tubes stuffed with a whitish to light brown substance, 135–195 μ in diameter, or 3–5 per mm. Dissepiments 50–100 μ thick, equal, edges concolorous, at apex velutinate. *Context* dark brown, upto 1 cm. thick, of interwoven hyphae. *Hyphae* dimitic both in the context and trama. Skeletal hyphae bovista type, deep brown in a mass, brown to light brown individually, aseptate, freely branched, branching mostly dichotomous, and attenuated to fine threads terminally, 2.2–4 μ wide, thick-walled, wall 0.8–1.2 μ thick, sometimes much more thickened so as to obliterate the lumen completely, take the stain. Generative hyphae hyaline, septate, sparsely branched, 1.6–2.6 μ wide, thin walled, take the stain. *Basidiospores* deep brown in a mass, brown individually, ellipsoid-obovate, rounded at both ends, sometimes appearing truncate at the narrow end, wall dark and thick, smooth aguttate, 7.2–11.2 x 4.4–5.6 μ. (Text-Fig. 3, A-D.)



Text—Fig. 3. *Ganoderma applanatum* var. *laevisporum* Humphrey,
A. Skeletal hyphae, x 750.
B Generative hyphae, x 750.
C. Smooth-walled basidiospores, x 1150. D. Pores, x 20.

Collected on living deciduous tree of *Quercus incana* Roxb., Sarkanda, Mussoorie, September 12, 1954, **198**. New record in India.

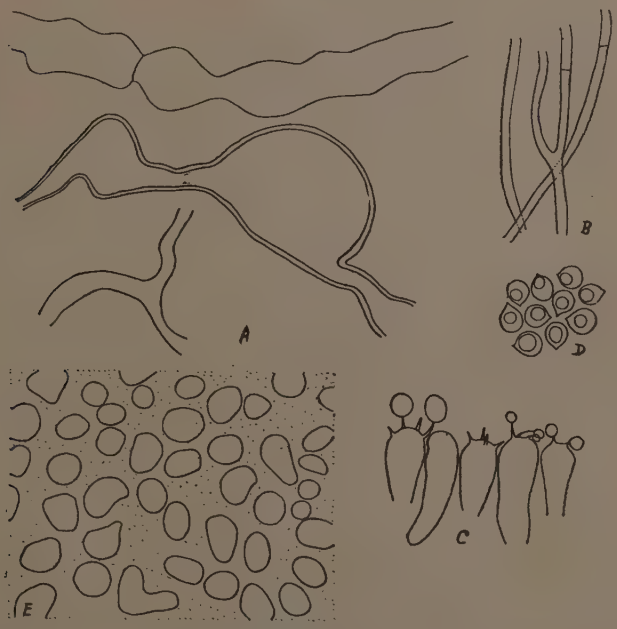
This Mussoorie collection shows close resemblance with *Ganoderma applanatum* (Pers. ex Wallr.) Pat. (= *Fomes applanatus* (Pers. ex Wallr.) except for its remarkable character of smooth spores, hence the variety *laevisporum* proposed by Humphrey (1931).

4. *Polyporus confluens* Alb. & Schw. ex Fr., Syst. Myc. **1** : 355. 1821.

Syn: *Boletus confluens* Alb. & Schw., Consp. Fung., p. 244. 1805.
Scutiger laeticolor Murr., Torrey Bot. Club Bul. **30** : 428. 1903.
Scutiger Whiteae Murr., Torrey Bot. Club. Bul. **30** : 432. 1903.

Hymenophore annual, solitary or caespitose, fleshy, and brittle when fresh, hard and somewhat brittle on drying, laterally stipitate. Stipe bright yellow, solid, unbranched, glabrous, cylindrical, sometimes slightly

compressed, 2.5–3.5 cm. long and up to 1.5 cm. thick. *Pileus* plane, fan shaped, profusely lobed, lobes very irregular, small to large, often overlapping, often confluent, 2.5–7 x 2.5–9 x 0.4–1 cm., surface bright yellow, turning dark brown or brown and often black spotted on drying, rough, covered over with abundant, prominent scales (or squamules), azonate, cuticle absent, hairs absent. Margin acute, highly lobed to deeply cleft, concolorous, incurved. *Hymenial surface* plane to silghtly convex, white, turning dark brown on drying, even, margin concolorous, fertile, decurrent. Dissepiments fimbriate. *Pores* not in strata, rounded, angular, to irregular, white in section, tubes separable from the context, up to 1 mm. deep, sometimes straight, mostly oblique so that the pores often become irregularly elongated, 150–270 μ in diameter, or 2–3 per mm. Dissepiments 60–195 μ thick, equal, of parallel hyphae, edges concolorous, at apex not velutinate, at apex fimbricate. *Context* white, turning dark brown to resinous black, shrinking much and becoming hard on drying, up to 8 mm. thick, of interwoven hyphae. *Hyphae* monomitic, context hyphae differ from the tramal hyphae as regards diameter and inflation. Context hyphae broad, hyaline, septate, much branched, thin walled, not



Text—Fig. 4. *Polyporus confluens* Alb. & Schw. ex Fr.
A. Context hyphae, x 750.
B. Tramal hyphae, x 750.
C. Basidia, x 1150.
D. Uniguttulate basidiospores provided with apiculus, x 1150.
E. Pores, x 20.

clamped, very irregular, highly inflated, inflated portions discontinuous so that the context hyphae aquire a highly lobed or beaded appearance, 2.4–5.6 μ wide but up to 30 μ in diameter in the inflated portions. Tramal hyphae long, hyaline, septate, branched; thin walled, narrower, uninflated, 1.6–3.2 μ wide, *Basidia* clavate type, clavate, light brown, 10.4–19.2 x 3.2–5.6 μ. Sterigmata 4, straight, curved, 0.8–2.4 μ long. *Basidiospores* hyaline globose to sub-globose or ovoid, apiculate, apiculus very fine and distinct, smooth, uniguttate, guttule large, filling one half or more, of the spore cavity, 2.8–4 x 2.4–3.2 μ. (Plate 1, Fig. 1; Text-Fig. 4, A-E.)

Collected on soil under Oak (*Q. incana*) forest, Chakrata Toll, Mussoorie, August 28, 1954, **199**. New record in India.

This fungus undoubtedly belongs to *Polyporus confluens* Alb. & Schw. ex Fr. and is easily recognized by its laterally stipitate, solitary to caespitose, bright yellow, fleshy fructifications, irregularly and profusely lobed, often confluent pilei, decurrent white hymenium turning dark brown on drying or becoming so in the herbarium, and small, ovoid, apiculate, uniguttulate basidiospores, 2.8–4 x 2.4–3.2 μ. The spores of the Mussoorie Collection are somewhat more ovoid than reported for the species.

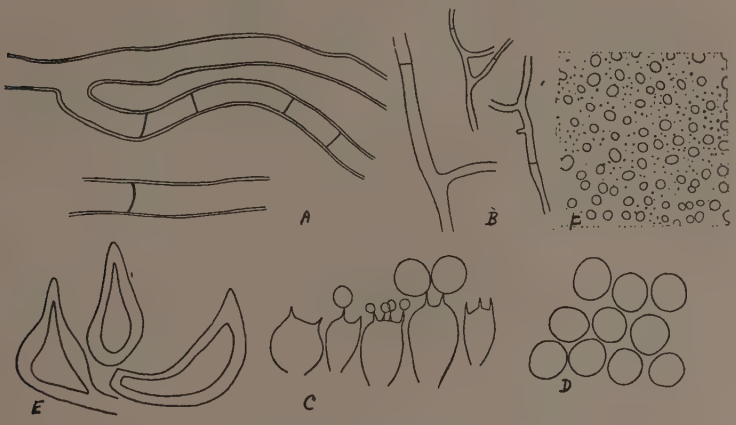
5. *Polyporus dryadeus* Pers. ex. Fr.

var. *brevisporus* var nov.

Hymenophori 8–24 x 7–18 x 2.5–7 cm., solitarii vel imbricati: pileo applanatus, fuscus: sporae breves, globosae, subgoblosae, vel ovoideae leves, hyalinae, 4.4–5.6 x 3.6–5.2 μ.

Hymenophore annual, solitary, or imbricate, tough and brittle when fresh, woody and brittle on drying. *Pileus* fan shaped, applanate, may be narrowed down into a stem like base or not, 8–24 x 7–18 x 2.5–7 cm., surface dark brown, not fading on drying, rough, somewhat concentrically zonate, zones of broader light brown and narrow dark brown colours, zones becoming indistinct on drying, cuticle present in the form of a thin crust which is easily indented, crust cracks and separates out on drying, hairs absent. Margin acute, or sub-acute, rounded in young specimens, entire, concolorous with the pileus, rarely deeply cleft. *Hymenial surface* plane to convex, brown, lighter than the pileus, even, margin white, turning darker on drying, with sterile margin, 0.7–1 cm. wide. Dissepiments not toothed. *Pores* not in strata, rounded, brown in section, up to 6 mm. deep, straight, 72–110 μ in diameter, or 5–7 per mm. Dissepiments 28–90 μ thick, equal, of compact parallel hyphae, edges of dissepiments not concolorous but lighter coloured, at apex velutinate. *Context* brown, 0.5–4 cm. thick, of interwoven hyphae. *Hyphae* dimitic both in the context and trama. Skeletal hyphae long, deep brown in a mass, individually yellowish brown to brown, septate, branched, 3.2–6.7 μ wide, thin walled, wall darker, do not take stain. Generative hyphae hyaline, septate, branched, 1–2.6 μ wide, thin walled, take the stain, hyphae at the edges of dissepiments are hyaline, rounded at tips and take stain. *Setae* abundant, ventricose, deep brown to reddish brown, 9.6–18.4 x 4.8–15.2 μ, not embedded, projecting 7.2–9.6 μ beyond the hymenial surface. *Basidia* clavate type, clavate, short, subhyaline, 4–9.6 x 4–6.8 μ Sterigmata 4, straight, 1.2–2 μ

long. *Basidiospores* hyaline, subglobose to globose, or ovoid, smooth, 4.4–5.6 x 3.6–5.2 μ (agguttate?). (Pl. I, Fig. 3 Text-Fig. 5, A-F.)



Text—Fig. 5. *Polyporus dryadeus* Pers. ex Fr. Var. *brevisporus* var. nov.,
A. Skeletal hyphae, x 750.
B. Generative hyphae, x 750.
C. Basidia, x 1150.
D. Subglobose to globose or ovoid basidiospores, x 1150.
E. Setae, x 1150.
F. Pores, x 20

Collected on stumps, and trunks of living trees of *Quercus incana*, The Park, Mussoorie, August 26, 1954, **200**.

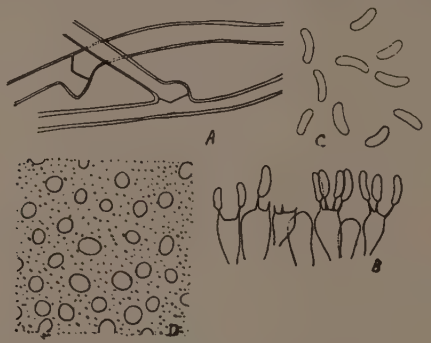
This Mussoorie collection resembles *Polyporus dryadeus* Pers. ex Fr. (Syst. Myc. 1: 374. 1821) in all respects except that its spores are conspicuously smaller for this species. The spores of Mussoorie collection measure 4.5–6 x 4–5 μ or mostly 5-6 μ while those of the type species are reported as 6–9.5 x 6–8 μ. According to Reid (Personal correspondence, 1957) there are a number of collections from India at Kew, which all agree in this character. As also suggested by him, this Mussoorie collection (n. 200) made here is a new variety of *P. dryadeus* Pers. ex Fr. and the varietal name *brevisporus* is proposed for it on account of its conspicuously smaller spores.

6. *Polyporus lacteus* Fr., Syst. Myc. **1** : 359. 1821.

Syn: *Leptoporus lacteus* (Fr.) Quel., Fl. Myc. p. 385. 1888.
Tyromyces lacteus (Fr.) Murr., N. Am. Fl. **9** : 36. 1907.

Hymenophore annual, solitary, or sometimes imbricate, dimidiate, soft and fragile, firm and brittle on drying. *Pileus* fan shaped, applanate, 1–4 x 2.5–7.5 x 0.7–1.7 cm., surface white, rough, finely tomentose, azonate, cuticle absent, hairs soft. Margin thin, acute, not abruptly thin, entire, white, concolorous with the pileus. *Hymenial surface* convex, snow

white, concolorous, with narrow sterile margin, up to 1 mm. wide. Dissepiments not toothed. *Pores* not in strata, rounded, white in section, up to 5 mm. deep, straight, sometimes oblique, 97–142 μ in diameter, or 4–5 per mm. Dissepiments 37–105 μ thick, equal, of compact parallel hyphae, at apex velutinate, edges concolorous. *Context* white, concolorous with the tubes, 0.5–1 cm. thick of interwoven hyphae. *Hyphae* monomitic both in the context and trama, long, light brown in a mass, hyaline individually, septate, branched, 2.8–5.6 μ wide, thin walled, clamped, clamp connection abundant and present at almost all septa. *Basidia* clavate type, oblong, short, subhyaline, 4.8–8 x 2.4–4.4 μ. Sterigmata 4, curved, 1.2–2.4 μ long. *Basidiospores* hyaline, typically allantoid, smooth, aguttate, 3.5–4.5 x 0.8–1.2 μ. (Pl. 1, Fig. 4. Text-Fig. 6, A-D.)



Text—Fig. 6. *Polyporus lacteus* Fr.,
A. Clamped hypha, x 750.
B. Mature basidia with basidiospores, x 1150.
C. Allantoid basidiospores, x 1150.
D. Pores, x 20.

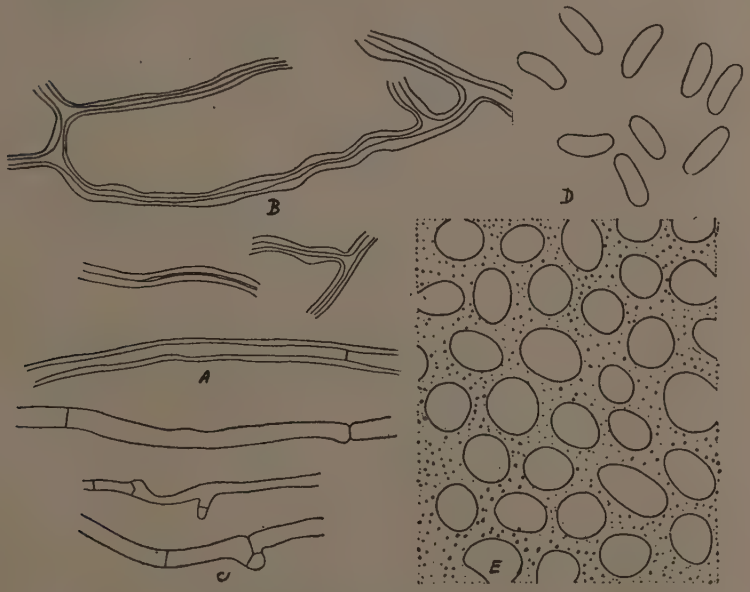
Collected from Dhanolti forest, Mussoorie, on stumps of *Pinus* species, September 11, 1954, **201**. New record in India.

This species is characterized by usually solitary, dimidiate, soft hymenophores turning firm and brittle on drying, thick, white and finely tomentose pileus lacking any cuticle, white context, monomitic and clamped hyphae, and typically allantoid basidospores, 3.5–4.5 x 0.8–1.2 μ.

7. *Trametes corrugata* (Pers.) Bres.

Hymenophore annual, mostly resupinate, slightly effused-reflexed, coriaceous when fresh, rigid and firm on drying, easily separable from the substratum when fresh, not separable on drying. *Pileus* effused reflexed laterally confluent, 0.5–1.5 x 1–4 x 0.1–0.5 cm., surface chestnut brown, mostly with white periphery, smooth, or rough, inconspicuously concentrically zonate, cuticle absent, hairs absent,but inconspicuously pubescent in the white peripheral region. Margin thin, acute, entire, white, turning, cream coloured on drying. *Hymenial surface* plane, dull white, turning light brown on drying, even, or uneven, margin concolorous, with sterile margin, 0.5–2 mm. wide. Dissepiments not toothed. *Pores* not in strata,

mostly round to oval or angular when usually polygonal to hexagonal, white in section, upto 2.5 mm. deep, mostly straight, sometimes oblique, sunken to uneven distances in the context, 192–265 μ in diameter, or 2–3 per mm. Dissepiments 90–300 μ thick, equal, of interwoven hyphae, at apex velutinate. *Context* white, 1–4 mm. thick, of interwoven hyphae. *Hyphae* trimitic within the context and trama. Skeletal hyphae long, hyaline to subhyaline, usually aseptate, sometimes septate, occasionally branched, 2.4–4.8 μ wide, thin to thick walled, wall upto 1.4 μ thick, lumen up to 3.2 μ wide, do not take stain. Binding hyphae bovista type, hyaline to subhyaline, aseptate, branched, 1.6–2.4 μ wide, thick walled, wall up to 0.8 μ thick, lumen up to 1 μ wide, do not take stain. Generative hyphae, hyaline, septate, branched, 1.6–4 μ wide, thin walled, clamps not common, take stain. *Hyphal pegs* columnar, yellowish brown, up to 48 μ broad, projecting up to 40 μ beyond the hymenial layer. *Basidia* clavate type, clavate to elongated, subhyaline to light brown, 16–20 x 3.5–5.4 μ Sterigmata 4, curved, 2.8–4.4 μ long. *Basidiospores* hyaline, cylindrical, or cylindric–ellipsoid, smooth, aguttate, 7–9 x 2.2–3.5 μ. (Pl. I, Fig. 5., Text Fig. 7, A-E.)



Text—Fig. 7. *Trametes corrugata* (Pers.) Bres.
A. Skeletal hyphae, x 750.
B. Binding hyphae, x 1150.
C. Generative hyphae with clamps, x 750.
D. Cylindrical to cylindric-ellipsoid basidiopores, x 1150.
E. Pores, x 20.

Collected on logs of deciduous wood and dead trees of *Psidium guajava* L., Nala Pani, Dehra Dun, August 3, 1953, **202**. New record in India.

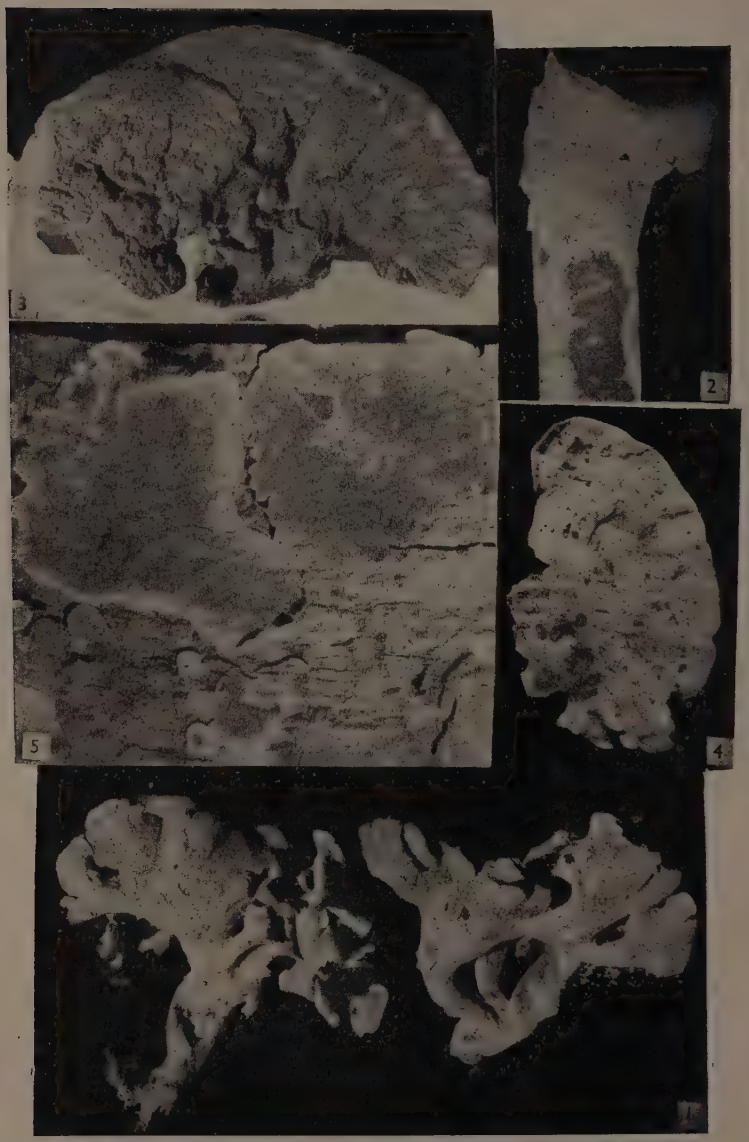


Fig. 1. *Polyporus confluens* Alb. & Schw. ex Fr.
Fig. 2. *Poria versipora* (Pers.) Baxter.
Fig. 3. *Polyporus dryadeus* Pers. ex Fr. var. *brevisporus* var. nov.
Fig. 4. *Polyporus lacteus* Fr.
Fig. 5. *Trametes corrugata* (Pers.) Bres.

This fungus undoubtedly belongs to *Trametes corrugata* (Pers.) Bres. and is characterized by the effused-reflexed, resupinate for the most part, hymenophore easily separable from the substratum when fresh, chestnut brown pileus with a white periphery, white context, trimitic hyphal system and cylindrical basidiospores, 7–9 x 2.2–3.5 μ.

8. *Trametes crenulata* Berk. Hooker's Jour. Bot. & Kew Garden Miscellany **6** : 164, 1854.

Hymenophore annual, sometimes biennial when new year's growth is formed on last year's pilei which had turned greenish due to some algal growth, usually imbricate, sometimes solitary, dimidiate, tough and leathery when fresh, hard and woody on drying. *Pileus* fan shaped, sometimes circular, applanate, 4.5–12 x 7–22 x 1.5–3.5 cm., mostly overlapping and confluent, or distinct, surface white or cream coloured, darkening on drying, somewhat uneven, concentrically zonate, cuticle absent, pubescent, hairs white. Margin acute, entire or wavy, concolorous, turning yellowish brown on drying. *Hymenial surface* convex, white or cream coloured, turning light brown or brown on drying, smooth, even, margin concolorous, with a narrow sterile margin, 1–1.5 mm. wide. Dissepiments not toothed. *Pores* not in strata, angular, mostly radially rectangular, usually circular near the margin, and sometimes daedaloid towards the base, lined by white pubescence, up to 1.5 cm. deep, sunken to uneven distances in the context, 270–360 μ broad, or 1–2

Text—Fig. 8. *Trametes crenulata* Berk.,
A. Skeletal hyphae, x 750.
B. Binding hyphae, x 750.
C. Generative hyphae, x 750.
D. Basidia with basidiospores, x 1150.
E. Anuglar to daedaloid pores, x 20.

per mm. in transverse direction. Dissepiments 165–330 μ thick, equal, of interwoven hyphae, at apex velutinate. *Context* white or cream coloured, upto 2 cm. thick, of interwoven hyphae. *Hyphae* trimitic both in the context and the trama. Skeletal hyphae long, subhyaline, aseptate, unbranched, sometimes branched, 4–7.2 μ wide, thin to thick walled, wall up to 2 μ thick, sometimes highly thickened so as to obliterate the lumen completely, do not take stain. Binding hyphae of bovista type, subhyaline, aseptate, branched, 2–3.6 μ wide, thick walled, thickening small to high so as to obliterate the lumen completely, do not take stain. Generative hyphae hyaline, aseptate, branched, 1.5–3 μ wide, thin walled, take stain. *Pseudocystidia* common, represented by protruding long skeletal hyphae, projecting up to 60 μ beyond the hymenial layer. *Hyphal pegs* occasional, light brown, 10–48 μ broad, columnar, projecting up to 34 μ beyond the hymenial layer. *Basidia* clavate type, clavate, subhyaline, 8–12 x 2.4–3.6 μ. Sterigmata 4, straight or curved, 1.6 – 3.2 μ long. Basidiospores hyaline, globose to subglobose, smooth, aguttate, very small, 1.2–2.4 μ in diameter. (Text-Fig. 8, A-E.)

Collected on stumps and living trees of *Q. incana*, the Park, Mussoorie, August, 1952, September 11, 1953. **203.**

This species is characterized by usually imbricate, dimidiate hymenophores, white and concentrically zonate, pubescent pileus, angular to circular to daedaloid pores, white context, trimitic hyphal system, very small, globose to subglobose basidiospores, 1.2–2.4 μ in diameter. The Mussoorie fungus is distinctly annual to biennial when next year's growth is formed on the first year's pileus which had turned greenish due to an algal growth.

Summary

Eight species of Polyporaceae collected from the Mussoorie Hills are described here. Out of these five are new records for India while var. *brevisporum* of *Polyporus dryadeus* Pers. ex Fr. is described here as a new variety.

Type collections have been deposited in the Herbarium of the Panjab University. Duplicate material is in the Herbarium, Royal Botanic Gardens, Kew, England.

Acknowledgments. The authors are deeply indebted to Dr. D. A. Reid, Royal Botanic Gardens, Kew, England, for help in the identification of the species and Prof. P. N. Mehra for providing facilities and encouragement. They are also thankful to Rev. Father Santapau, St. Xavier College, Bombay, for help in the latin diagnosis of the new variety.

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PRELIMINARY STUDIES ON UROMYCES DECORATUS, THE CAUSAL ORGANISM OF SANNHEMP RUST

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Introduction: Sannhemp (*Crotalaria juncea* Linn.) is an important crop of our country as it is used either for green manuring or as a fibre crop. As a fibre crop it is ranked next to jute. Sannhemp fibre is essentially a cordage fibre. One of the important diseases on the crop that damages the fibre is rust, caused by *Uromyces decoratus* Syd. The rust is common throughout India and was first reported by Butler (1918). In Delhi area the rust makes its appearance sometimes in September or October though disease has, at times, been reported as early as the 4th week of August. The sori appear in all the aerial parts of the plant including pods though they normally remain restricted to leaves and stems. The rust appears first as small specks and later develops into big linear dark brown coalescing pustules. Quite often these pustules get surrounded by small secondary pustules in the form of uniform rings. With advancement in age,the uredosori are gradually replaced by teleutosori which are black in colour. Although the rust has been reported as early as 1918 in India, there is no information regarding the perpetuation of the disease from season to season.

Under Delhi conditions sannhemp is usually sown in June or early July before the first monsoon shower and the plants are ploughed down before *rabi* sowings. The plots kept for seed purposes are normally harvested in December to February when the teleutospores of the rust are very common.

Experimental: The uredosori of the rust are found sub-epidermally and the epidermis gets ruptured when they attain maturity. The spores have a hyaline pedicel, supporting the globose uredospores. The spores are sparsely echinulate, brown in colour with pigmented walls. The echinulation is more in the central portion gradually diminishing towards periphery. Each spore usually has four germ-pores but in some the number is 5 to 6 or even 7. The uredosori are devoid of paraphyses.

The uredospores germinate readily between 5 °C and 30 °C in a drop of water with optimum at 18–25 °C. At 5 °C and 10 °C the initiation of germination was observed in 6–8 hours whereas at higher temperatures it was noticed in less than 4 hours. Stored at 5 °, 20–25 ° and 35 °C after air drying, the spores remained viable for 4 months, 40–45 days and 10–12 days respectively. At 45 °C dry-heat the spores were killed in 72–96 hours whereas the spores kept in a humid chamber at that temperature lost their viability in less than 4 hours.

Culture of the rust could not be maintained at Delhi during May to July when tried for three successive years although the host could be grown throughout the year.

Sannhemp sticks showing uredospores collected in March from artificially inoculated plots were kept outside under a shady tree and in the varandah. Viability tests made from time to time showed that the material had lost its viability before the end of April or by the first week of May.

To determine if the rust could survive as a dormant mycelium during unfavourable weather conditions of summer, artificially inoculated plants showing good development of uredo pustules were kept outside in a shady place from April to July. The plants for this experiment were raised in February and inoculated in March. The viability of uredospores were tested from time to time. By the middle of May the uredospores had lost their viability and the further development of the rust ceased completely. At this stage all the infected portions were marked with India ink. The marking was done with a view to locate the portions where the dormant mycelium, if it had remained viable throughout summer months, would become active on return of favourable conditions to produce new uredospores. The uredo material from infected lesions was tested from time to time to establish if fresh uredospores have appeared. Even through the plants were kept under observations till end of September no fresh uredospores were found in the plant. If the mycelium were viable it could have formed uredospores sometimes in July because plants of Sannhemp inoculated with fresh uredospores in July and kept under natural conditions got infected proving thereby that conditions even in July were quite congenial for development of rust and, therefore, it is evident that the rust does not survive through summer months either as uredospores or even as a dormant mycelium.

The teleutospores of the fungus appear about a month or two after the appearance of the uredo stage and in the same sorus. The teleutospores are unicelled, pedicellate and erumpent. They do not require a resting period and germinate readily if temperature and humidity are suitable for their germination. The teleutospores collected from fields in January and February germinate readily in less than 48 hours producing a stout, septate promycelium (75 μ to 105 μ in length and 7–10 μ in breadth) and sterigmata measuring 12–15 μ by 6–9 μ. (Plate-1).

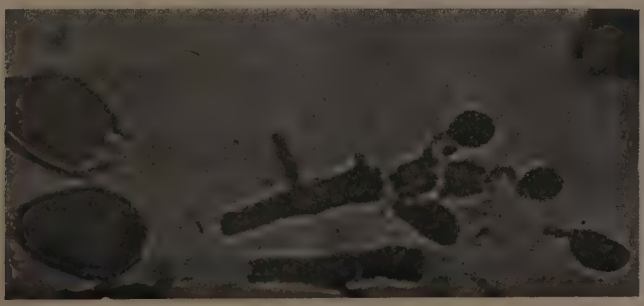

Plate-1. Germinating teleutospores of *Uromyces decoratus*.

Although the teleutospores do not require a resting period it was found that the spores collected at different times from fields took different periods to germinate. For instance freshly formed material collected from the fields in October or November took more than 144 hours to germinate whereas material collected from the same field in January and February germinated in less than 48 hours. There was, however, no significant difference in the percentage of germination as both the materials showed 95–98 per cent germination 36 hours after the initiation of germination. It was felt that this difference in the time required for germination of teleutospore might be due to the effect of exposure of spores under natural conditions for some times. To investigate the possible reasons for this behaviour, the teleutospores were raised in the glasshouse and collected 2 to 3 weeks after their formation. It was found that they took nearly a week for germination. The material was then divided into 3 lots and treated as below:—

(i) One lot was wrapped in moist blotting paper and kept in humid chamber and kept at 5 °C in frigidaire.

(ii) Second lot kept at 5 °C frigidaire wrapped in dry blotting paper.

(iii) Third lot kept inside the room at 20–25 °C. (Control).

The material was treated for 10 days and after the period, material i and ii were taken out and dried for 3 days and then both stored under dry conditions for another 10 days in frigidaire. After the treatments were over all the three lots were tested simultaneously at 25 °C.

The germination tests revealed that the teleutospores which had been soaked in water germinated more readily probably because of the absorption of water by then. It is possible, therefore, that teleutospores exposed to natural conditions in winter months, when there is plenty of moisture in the fields in the form of dew, absorb a certain amount of water which is required for their germination. This accounts, to a certain degree, for the quicker germination of teleutospores collected in January as compared to these which were collected immediately after their formation in October. It is well-known that alternate wetting and drying improves the germination of teleutospores of several rusts (Prasada 1948).

Certain abnormalities in the germination of teleutospore were noticed while testing the viability of the material. Usually the teleutospores germinated without any resting period, producing a stout promycelium with four sporidia. If, however, conditions were not normal, particularly if temperature was high, there was a tendency on the part of sporidia to bud out 'secondary sporidia' and develop into a chain while still attached to the promycelium. At times a chain of 3-4 sporidia might be formed. These sporidia, however, get easily detached with slightest disturbances. The phenomenon of formation of secondary sporidia is not rare and according to Arthur (1929), has been observed even by many earlier workers such as Anton de Bary and Tulasne. Bessey (1952) has recorded that under conditions of extreme humidity "the primary sporidia and the succeeding ones of *Kunkelia nitens* (Schw.) Arthur, remain attached in chains of four or five successive smaller ones".

A few other abnormalities were also observed regarding formation of promycelia. In some cases the teleutospores were found to form two or even 3 promycelial structures from a common bulbose base. The promycelia of such structures always remained aseptate and sterile. In other cases abnormally long promycelia, measuring upto 175 μ to 200 μ were observed. In these some septation and formation of rudimentary sterigmata were observed but in all such cases no sporidia appeared.

The teleutospores germinated between 10 to 27 °C. though best germination was obtained between 22–25°C. At 10 C only 1-2 per cent teleutospores germinated and no germination was obtained below 8 °C and over 28–30 C. The spores lost their viability totally in less than 12 days at 45°C but at the same temperature in moist heat the material was rendered inviable in 16–24 hours. Hence it was felt that the material might not be able to survive through summer months particularly if there were showers of rain during that period. To investigate further the actual role of teleutospores in tiding over summer months sannhemp sticks showing well developed teleuto pustules of the rust were treated in the following manner in the month of march:—

(1) Sannhemp sticks showing teleutospores, tied in bundles, were kept in:—

  (a) a shady place

  (b) in the sun

(2) Material stored in the frigidaire.

The viability of the material was tested from time to time at regular intervals. This experiment was repeated for three successive years and every year the material kept outside, either in the sun or in shade, lost its viability by the middle of May whereas the material stored in frigidaire remained viable for more than nine months.

From the evidence collected above it was felt that the rust cannot survive through teleutospores during summer months under Delhi conditions.

To establish autoecious or heteroecious nature of the rust, seedlings of sannhemp were inoculated with germinating teleutospores and kept in a humid chamber for 36 hours before transferring them to glasshouse benches. A series of inoculations were done in different glass-houses during different parts of the year to ensure a wide range of temperature but the plants did not get infected proving thereby that the rust is not autoecious.

To test the host range of the fungus some species of *Crotalaria* were raised under spore-proof conditions in a glasshouse and were inoculated by the uredospores, collected from sannhemp and *C. shrica*, *C. verrucosa*, *C. medicagenea*, *C. pumila* and *C. maxillaris* got infected while *C. retusa*, *C. stricta* and *C. intermedia* did not.

The rust has a fairly wide host range and can infect some of the cultivated and wild species of *Crotalaria*. Naturally infected plants of *C.*

medicagenea and *C. maxillaris* were also found infected in the experimental plots of Indian Agricultural Research Institute. The former was also found infected near about Delhi. Cross inoculation experiments proved that the rust from these could be successfully transferred to sannhemp and *vice versa*.

Discussion. From the above account it is evident that the rust cannot survive during summer months under Delhi conditions either as uredospores or teleutospores or even as a dormant mycelium. In these respects it resembles with wheat or linseed rusts which do not survive in the plains of India during summer months. It is quite possible that the rust can survive in the lower hills either in a regular crop or some hosts such as *C. medicagenea* which is reported to be perennial in the hills (Collett - 1921). It is, therefore, essential to carry out regular surveys in the hilly regions particularly in lower hills where sannhemp is cultivated either for green manuring or as a summer crop. Such surveys are likely to reveal the role of other species of *Crotalaria* as well as that of regular crop in the hills, in the perpetuation of the disease.

From the investigations carried out it appears that there is no local source of infection under Delhi conditions and the rust gets killed during summer months. The fact that rust usually does not appear before end of August, though conditions for its development are congenial even by the middle of July, support this view.

Summary

The uredospores germinate readily in water in less than 3 hours. Optimum temperature for their germination is 18–25 °C. The teleutospores do not require a resting period and usually germinate in less than 48 hours. Optimum temperature for their germination is 20–25 °C. The uredospores and the teleutospores cannot survive during summer months under Delhi conditions. The rust has been demonstrated to be not autoecious.

Since the rust has a wide host range, the possibility of its survival like wheat or linseed rusts, requires to be investigated. So far studies have shown that the rust cannot survive in the plains particularly, near about Delhi either as uredospores or teleutospores or even as a dormant mycelium on sannhemp during summer months.

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STUDIES ON CERCOSPORA CRUENTA OCCURRING ON VIGNA CATJANG

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Cercospora leafspots are of common occurrence on several leguminous plants. *Vigna catjang* Walp., a member of the family, cultivated either as a green manure or for its fruits, is also affected by some species of *Cercospora*. Ellis and Martin (1882) first recorded from U.S.A. a *Cercospora* with hyaline acicular conidia on *V. catjang* and named it as *C. canescens* Ell. & Mart. In 1880 Saccardo recorded another obclavato-cylindric form of *Cercospora* on this host and named it *C. cruenta* Sacc. and again in 1886 he reported *C. canescens* on the same host (Chupp 1953) Ellis and Everhart (1889) reported the occurrence of a similar species of *Cercospora* on the host and named it as *C. vignae* Ell. & Ev. A species with pale olivaceous obclavato-cylindric conidia, named *C. vignicaulis* Tehon has also been recorded on this host (Ellis and Everhart 1889). Thirumalachar and Chupp (1948) first observed the occurrence of *C. canescens* on *V. catjang* in India. Govindu and Thirumalachar (1958) also listed the occurrence of *C. canescens* on the host in India.

During December 1958 and January 1959 a severe Cercospora leafspot of *V. catjang* was observed in several fields in and around Chidambaram, S. Arcot, Madras State, where the crop is cultivated both for its fruits and as a green manure. Detailed studies were undertaken on this disease and the results are reported here.

Disease Symptoms and Identity of the Fungus: The fungus manifests itself on the leaves as minute brown spots with diffuse chlorotic halos. Soon they become necrotic and rusty brown in colour, the depth of which is more in the periphery than in the central region. They are irregular in shape, range from 1 to 16 mm in size and are mainly confined to the leaf blades. In general, 3 to 7 spots are found on each leaflet and they increase in size and coalesce in many cases resulting in the yellowing and withering of the leaflets. In a field the disease starts on isolated plants and soon spreads rapidly, causing wilting of large patches of the crop.

The fruiting and morphological characters of the fungus were found to be similar to those described by Saccardo for *C. cruenta* with the characteristic pale brown coloured conidiophores, which are attenuated with conical tip and obclavato cylindric conidia (Chupp 1953).

Pathogenicity and Host Range: Fresh leafspots on *V. catjang* were collected from the field and the fungus was brought into culture by the tissue culture technique, using yeast extract dextrose agar medium. Carrot leaf extract agar was found to be fairly good for growth and sporulation of the fungus and so it was used for maintaining stock culture of the

fungus. By repeated subculturing it was found to lose the sporulating capacity.

The pathogenicity of the fungus was tested by inoculating a week old culture of the fungus on the leaves of a month old plants of *V. catjang* in the usual way.

C. cruenta was readily infective on the leaves of *V. catjang* in all the inoculation tests, giving an average of 43 per cent infection. The symptoms of infection were similar to those occurring in nature, except for more intensive chlorotic halos around the spots. The fungus was reisolated from the infected spots and found to be identical with the original inoculum.

The fungus is highly specific to its natural host and failed to infect any of the Cucurbitaceous or Leguminaceous Plants tested, except *Dolichos lablab* L. to a very low extent. The symptoms of infection on *D. lablab* were rather different from those caused by *C. canescens* on the same host in natural condition, in that, the spots in the former case being rusty brown without any chlorotic halos as against the light brown spots with characteristic halos in the latter case.

Nutrient and Richard's agar media were not very favourable, while the others tested were uniformly good for the fungal growth. The fungus was also found to be capable of producing the characteristic pink soluble pigment in both the complex organic and synthetic media, though in the carrot leaf extract and carrot agar media no distinct pigment formation could be seen, because of the dark colour of the media.

The fungus could not grow well in nutrient broth and Richard's solution. Potato dextrose broth, among the complex organic media, and Czapek's solution, among the synthetic media tested, were found to be the best. The pigment production was in line with the observations made in the respective agar media.

The optimum pH for the growth of the fungus was around 6, though the fungus could grow fairly well in the pH range of 4 to 9.

Irrespective of the initial pH level,the fungal growth tended to move the final pH of the medium towards neutrality; the acid media became almost neutral and the alkaline media became much less alkaline within 12 days.

The fungus seems to grow best both in the liquid and agar media in the temperature range around 25°C; there was some growth at 18° and 31°C, practically little or no growth at 8° and 38°C.

The fungus could grow equally well at the concentrations of 1.5 and 3.0 per cent sugar content in the medium, irrespective of the variations in the quantity of $NaNO_3$ added. But at 4.5 and 6.0 per cent sugar content there was better growth, irrespective of the quantity of nitrogen

added. The intensity of the pink pigment produced was also proportionately more with the increasing amounts of sugar added, but not with that of $NaNO_3$.

DISCUSSION: The present report on the occurrence of *C. cruenta* on this host appears to be the first record in India. In the detailed studies on species of *Cercospora* occurring in this locality made by the authors, it was observed that *C. cruenta* occurred on *V. catjang* only, whereas *C, canescens* was commonly found on *Dolichos lablab*, *Phaseolus mungo* L. and *P. aureus* Roxb. In the inoculation studies also the fungus was specific on *V. catjang* except for a mild infection on *D. lalblab*, whereon the symptoms of infection were much different from those of the natural infection of *C. canescens.*

Though over 1,500 species of *Cercospora* have been reported so far on various host plants, not many of them have been brought into pure culture on artificial media and their pathogenicity and host range studied. Because of the difficulties involved in bringing them into culture some workers have used the conidial suspensions from the host plant for inoculations (Jenkins 1938, Thirumalachar 1953). The difficulties in bringing species of *Cercospora* into culture have been stated by some workers (Singh 1931, Jenkins 1938, Shanta 1953). This appears to be due to the highly exacting nutritional requirements of the fungus. In the present investigations, though some difficulty was experienced in bringing into culture the fungus from the host tissue on most media, it was readily brought into culture on yeast extract dextrose agar, which might be due to the richness in vitamin content of the medium.

The comparative studies on growth of the fungus on various media indicate that it is capable of growing equally well on both synthetic and complex organic media. Czapek's agar, which is a rich medium containing relatively more carbohydrate and more minerals, was found to be better than Richard's medium which has a poor carbohydrate source. Similarly, among the complex organic media, potato dextrose agar medium which is rich in starch was found to be the best. These results indicate that the fungus favours a medium rich in carbohydrate source. Further proof to this belief was obtained in the studies on the influence of carbon/nitrogen ratio on the growth of the fungus; increased concentrations of the carbon source induced better growth of the fungus, but not that of the nitrogen source.

The fungus was found to grow best around pH 6, though it could grow fairly well in the wide range of pH 3 to 9. The sap from the leaves of *V. catjang* was tested and found to be pH 6.4 and this indicates the suitability of host plant for the fungus to thrive well. The optimum temperature for the growth of the fungus was around 25 °C. This appears to be the case for some other species of the genus studied (Kiru and Fukj 1936, Tasugi and Ikeno 1956).

Since the fungus appeared to lose its sporulating capacity by repeated subculturing, no conclusive evidence could be obtained on the influence of nutritional and physiological factors on its sporulating capacity and more detailed studies in this line are needed.

SUMMARY

A leafspot disease of *Vigna catjang* Walp., caused by *Cercospora cruenta* Sacc., being recorded for the first time in India, was studied in detail. The fungus was brought into pure culture and its pathogenicity and host range examined. In artificial inoculations it was found to readily infect leaves of *V. catjang* and to a mild extent on *Dolichos lablab* L. Otherwise the fungus was highly restricted in its host range.

Among the eight synthetic and complex media tested for comparative growth of the fungus, Czapek's and potato dextrose media were found to be most suited. Evidence was obtained to indicate that the fungus favoured a medium rich in carbohydrate for its growth. An incubation temperature around 25°C and the pH of the substratum around 6.0 were found to be the optima for the growth of the fungus.

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BOOK REVIEW

A revised list of Plant Diseases in Tanganyika territory. By E. A. Riley—Mycological Paper No. 75, Commonwealth Mycological Institute, Kew, Survey 1960 pp. 42. **Price 10s.**

This is a comprehensive list of Plant Diseases occurring in Tanganyika territory (East Africa) based chiefly on the lists already published by Wallace and Wallace (Mycological Papers No. 26 and 51). This brings together under one cover records of various plant diseases occurring in that region, thus making the list up-to-date and provides brief annotated account of those that are economically important or the control measures adopted against them where known. There is a slight departure from the earlier list in that the host plants are arranged alphabetically under the latin names rather than the popular names which vary from one place to another. Although the author has tried to provide latest valid names for the pathogens, yet some workers on nomenclature of fungi may not agree with him in the use of such names as *Corticium solani* (Prill. & Delacr.) Bourd. & Galz. and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. for which they might have preferred *Pellicularia filamentosa* (Pat.) Rogers and *Glomerella cingulata* (Stonem) S. & V. S. respectively. In the index provided by Wallace and Wallace, only fungi or bacteria had been included the present list provides index for all pathogens and even physiological disorders. The introductory part has been considerably expanded to include Geo-physical characters of the region, which gives the readers an opportunity to know the conditions under which these diseases occur.

This is a very useful list and will be highly appreciated by the plant pathological workers all over the world, in particular those working in the tropical countries.

R. L. Munjal

"Potato in West Bengal" By H. C. Choudhuri—Government of West Bengal, Calcutta. 125 pages, 1957.

"Potato in West Bengal" by H. C. Choudhuri is a useful Handbook particularly for the potato growers of West Bengal, for whom the book appears to have been written. The author from his personal knowledge of the crop and his experience as a research worker for several years has succeeded in putting before the reader a vivid picture of the potato growing in the State and some of the problems connected with it.

The first three chapters of the book deal with the information on the acreage and yield of potato in different districts, the varieties grown and a brief account of the agricultural practices and the problems connected

with the storage. A general description of the diseases and pests and of a few standard varieties has been provided. Also results achieved in the State as a result of experimentation on spraying against potato blight, hormone therapy control of diseases and pests in storage, fertiliser and varietal trials have been described. Information on the local marketing practices and disposal of the produce has been included to give some idea about the economics of potato growing in the state. It appears, however, that the editing has not been done with the care needed in such cases as also some of the scientific names of the causal organisms of diseases are outdated. The occurrence of the two bacterial diseases i.e. Black leg and Ring Rot of potatoes in India has been a disputed question and the report of these diseases from West Bengal would require to be carefully checked up.

R. S. VASUDEVA

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Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), p.p. 38-41 and Riker's "The preparation of manuscripts for Phytopathology, *Phytopathology* 36 : 953-977, 1946, before preparing their mss. and figures.

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